Effect of phytase supplementation on rumen fermentation characteristics and phosphorus balance in lactating dairy cows

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Abstract

This study aimed to evaluate the effects of exogenous phytase on rumen fermentation characteristics, the phosphorus (P)-flow at the duodenum and the P-balance in lactating dairy cows. For this purpose ruminal and duodenally fistulated cows were assigned to one of three dietary treatments: high P (HP) diet (n=7) provided a total of 45 g/d of P, archived by a supplementation of dicalcium phosphate to the diet; low P (LP) diet (n=5) provided 34 g/d of P without supplementation; LP+phytase (LP+PHY) diet (n=5) provided 34 g/d of P supplemented with an exogenous phytase. Daily matter intake and milk yield were recorded daily. In the first week of a sampling period P-balance was determined. Samples of ruminal fluid were taken and duodenal chyme was collected in the second sampling week. Ruminal pH and the concentration of volatile fatty acids were not different between the treatments. The HP-group shows a higher P-flow at the duodenum than other groups. No differences in apparent total tract P-digestibility were found between the treatments. The HP-group had a higher P-flow at the duodenum than other groups. No differences in apparent total tract P-digestibility were found between the treatments. The HP-group showed a higher P-flow at the duodenum and the P-balance in lactating dairy cows.

Materials and methods

Animal treatments and experimental design

The study was conducted in accordance with the German Animal Welfare Act with the approval of the Lower Saxony State Office for Consumer Protection and Food Safety, Oldenburg, Germany. The experiment with a total of nine multiparous German Holstein dairy cows was carried out at the experimental station of the Institute of Animal Nutrition, Friedrich-Loeffler-Institute (FLI), Federal Research Institute for Animal Health, in Braunschweig, Germany. The cows were fitted with large rubber cannulas in the dorsal sac of the rumen (inner diameter: 10 cm) and T-shaped cannulas at the proximal duodenum close to the pylorus (inner diameter: 2 cm). At the beginning of the trial the average milk yield of the cows was 20.6±0.2 kg/d, the animals had an average body weight of 558±57 kg and the animals were on average in their 3.6±1.4 lactation. The cows were kept in a tethered stall with neck straps and individual troughs with free access to water. Cows were milked daily at 5:30 and 15:30 h.

The cows received three diets differing in the concentration of P and phytase supplementation. The P concentration of the high P (HP) diet was calculated to cover the recommendations for a dairy cow with a milk yield of 20 kg/d and a feed intake of 16 kg dry matter (DM)/d as given by the German Society of Nutrition Physiology (GfE, 2001). The basal low P (LP) diet was intended to contain 80% (2.6 g P/kg DM) of the P of the diet for group HP (3.3 g P/kg DM). The animals of group LP+phytase were supplemented with an exogenous phytase. Dry digestible (LP+PHY) diet (n=5) provided 34 g/d of P supplemented with an exogenous phytase.

Consequently a phytase supplementation could increase P-supply to the microbes. An insufficient P-supply to the microbes reduces organic matter (OM) fermentation and microbial protein synthesis rates in the rumen (Kincaid and Rodehutscord, 2005). We hypothesized that exogenous phytase increases the P-supply to the cow and rumen microbes, caused by increased ruminal degradation of InsP6, in dairy cows fed a highly digestible diet. For this purpose, two P-reduced diets, one of them supplemented with exogenous phytase, were compared to a diet supplemented with dicalciumphosphate to meet the P requirement for dairy cows and rumen microbes. The objective of the experiment was to examine the effects of exogenous phytase on rumen fermentation characteristics, the P-flow at the duodenum and the P-balance in lactating dairy cows.

Introduction

Phytase releases phosphorus (P) from phytate [myo-inositol hexakisphosphate (InsP6)] and lower inositol phosphates by dephosphorylation and hydrolysis (Suttle, 2010). In ruminants the microbial community produces phytase which is responsible for InsP6 degradation (Morse et al., 1992). A study by Clark et al. (1986) pointed out that 98% of dietary InsP6 was hydrolysed to inorganic P (P_i) in the gastrointestinal tract of dairy cows. However, in vitro investigations by Godoy and Meschy (2001) showed that in specific situations the ruminal phytase hydrolyses not all P from InsP6. They carried out an experiment with a semi-continuous culture system, infusing P_i or a phytate source into the system. The results showed that only 67% P from the phytate source were available. These results are in contrast to the mentioned study of Clark et al. (1986) and were supported by an in vivo study by Kincaid et al. (2005), which showed values of phytate hydrolysis of approximately 80% irrespective of the dietary grain sources barley and corn. In both dietary situations (26% barley or 26% corn in the diet) an exogenous phytase supplementation increased the InsP6 hydrolysis from approximately 80 to 85% (Kincaid et al., 2005). We hypothesized that exogenous phytase increases the P-supply to the cow and rumen microbes, caused by increased ruminal degradation of InsP6, in dairy cows fed a highly digestible diet with increased passage kinetics based on corn silage (70%) and concentrate (30%).

For this purpose, two P-reduced diets, one of them supplemented with exogenous phytase, were compared to a diet supplemented with dicalciumphosphate to meet the P requirement for dairy cows and rumen microbes. The objective of the experiment was to examine the effects of exogenous phytase on rumen fermentation characteristics, the P-flow at the duodenum and the P-balance in lactating dairy cows.

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The cows received three diets differing in the concentration of P and phytase supplementation. The P concentration of the high P (HP) diet was calculated to cover the recommendations for a dairy cow with a milk yield of 20 kg/d and a feed intake of 16 kg dry matter (DM)/d as given by the German Society of Nutrition Physiology (GfE, 2001). The basal low P (LP) diet was intended to contain 80% (2.6 g P/kg DM) of the P of the diet for group HP (3.3 g P/kg DM). The animals of group LP+phytase...
Table 1. Ingredients of concentrates and chemical composition of the diet components used during the trial.

| Variable | Corn silage | HP | LP | LP+PHY |
|----------|-------------|----|----|--------|
| Ingredients, % | | | | |
| Corn | 35.0 | 35.0 | 34.2 |
| Wheat gluten | 10.0 | 10.0 | 10.0 |
| Dried sugar beet pulp | 48.0 | 48.5 | 48.5 |
| Urea | 3.0 | 3.0 | 3.0 |
| Sodium chloride | 0.2 | 0.2 | 0.2 |
| Mineral premix 6 | 2.0 | 2.0 | 2.0 |
| Premix with phytase 8 | - | - | 0.83 |
| Dicalcium phosphate | 1.8 | - | - |
| Calcium carbonate | - | 1.3 | 1.3 |
| Chemical composition, g/kg DM | | | | |
| Nutrients | | | | |
| OM | 955 | 915 | 917 | 924 |
| CP | 83 | 258 | 254 | 256 |
| EE | 32 | 28 | 27 | 26 |
| ADF | 263 | 112 | 118 | 120 |
| NDF | 501 | 265 | 269 | 280 |
| Minerals | | | | |
| P | 2.74 | 4.89 | 1.88 | 1.96 |
| InsP6 | - | 0.57 | 0.39 | 0.53 |

HP, high phosphorus (diet added with dicalcium phosphate); LP, low phosphorus (diet without phosphorus and phytase supplementation); PHY, phytase (diet without phosphorus-supplementation, but added with an exogenous phytase); DM, dry matter; OM, organic matter; CP, crude protein; EE, ether extract; ADF, acid detergent fibre; NDF, neutral detergent fibre; P, phosphorus; InsP6, myo-inositol hexakisphosphate. °Composition (per kg): calcium, 200 g; sodium, 120 g; magnesium, 40 g; vitamin A (E672), 1,000,000 UI; vitamin D3 (E371), 100,000 UI; vitamin E (alpha tocopherol acetate), 1500 mg; manganese [(manganese (II)sulphate, monohydrate E5], 5.4 g; zinc (zinc oxide) E6, 6 g; copper (copper sulphate pentahydrate E4), 1 g; iod (calcium iodate, waterfree E2), 100 mg; selenium (sodium selenate E8), 40 mg; cobalt (cobalt sulphate, monohydrate, E1), 25 mg. °Composition (per kg): 0.952 kg corn grain added with 0.048 kg experimental phytase.
Phytase in dairy cows

Detergent fibre (NDF) was conducted following the methods of VDLUFA (1997). Samples of morning and evening milk were pooled according to their milk yields and freeze dried for analysis of P. Faeces samples taken during the first experimental weeks were also freeze dried for the determination of nutrients. The phytase content of the concentrates was determined via the phytase activity and expressed as FTU/kg feed (Engelen et al., 1994). Phosphorus in diet, milk, urine and faeces was analysed with an optical emissions spectrometer with inductively coupled plasma ([ICP-OES] Quantima; GBC Scientific Equipment Pty Ltd, Braeside, Australia) according to VDLUFA (1997).

Myl-inositol hexakisphosphate was analysed in the feedstuffs, ruminal fluid, duodenal chyme and faeces using high-performance ion chromatography according to the method of Brejnholt et al. (2011). The InsP6 content in ruminal fluid, duodenal chyme and faeces was on a very low level. Therefore the determination of InsP6 in these samples was not possible and no data are available. Similar problems for analysis in digesta samples were reported by Brask-Pedersen et al., (2013). The milk samples were analysed for fat, protein, lactose, urea and the SCC with Fourier transform infrared spectroscopy and flow cytometric measurement system (Milkoscan FT 6000 combined with a Fossomatic 5000; Foss Electric, Hillerød, Denmark).

Volatile fatty acids (VFA) in rumen fluid were analysed using a gas chromatograph (HPS890II; Hewlett Packard, Böblingen, Germany) equipped with an automated injector (HP7673 II; Hewlett Packard), a flame ionization detector and an integrator (HP 3396 II; Hewlett Packard), a flame ionization detector and an integrator (HP7673 II; Hewlett Packard). For sample preparation samples were centrifuged at 40,000 g at 4°C. A self-packed glass column (length 1.8 m, inner diameter 2 mm) filled with Chromosorb WAW 80/100 mesh with 20% Neopentyl-Glycol-Succinate and two percent ortho-phosphoricacid (Analyt, Mülheim, Germany) was used for separation of VFA. Flow rates of the flame ionisation detector combustion gases hydrogen and synthetic air were 30 and 420 mL/min, respectively. Nitrogen was used as a carrier gas with a flow rate of 25 mL/min. Isothermal separation was carried out at an oven temperature of 130°C. The injection temperature was 220°C and the detection temperature 250°C.

Ammonia-N (NH3-N) in rumen fluid and duodenal chyme was analysed according to DIN 38406-ES-2 (Beuth, 1998). The following analyses were carried out in the freeze dried and ground duodenal samples. The DM and ash contents of duodenal chyme were analysed in the daily pooled samples with the same methods as the feedstuffs. The proportion of microbial-N of the non-ammonia-N (NAN) in duodenal chyme was estimated using near infrared spectroscopy according to Lebzien and Paul (1997). Cr2O3 in duodenal chyme was measured using an ICP-OES (Quantima) after sample preparation according to Williams et al., (1962). The chromium concentration was used to calculate the daily duodenal DM flow. According to the daily duodenal DM flows on the 5 sampling days, one aliquot pooled sample was generated per cow per 5 sampling days. In the pooled samples NDF and ADF were analysed by the same methods as the feedstuff.

Calculations

The metabolisable energy (ME) and net energy for lactation (NEL) content of the diets were calculated using the regression equations given by the GfE (2001). Gross energy (GE), crude protein (CP), ether extract (EE), nitrogen free extract (NFE) were obtained from analyses of the feedstuffs, while digestible EE (DEE), digestible crude fat (DCF) and digestible OM (DOM) were obtained from the digesta trial:

\[
\begin{align*}
\text{GE (MJ)} &= 0.0239 \times \text{CP} + 0.0398 \times \text{g} \\
\text{EE} &= 0.0201 \times \text{g} + 0.0175 \times \text{g} \\
\text{ME (MJ)} &= 0.0312 \times \text{g} + 0.0136 \times \text{g} \\
\text{DCF} &= 0.0414 \times \text{g} + 0.00234 \times \text{g} \\
\text{DOM} &= 0.0463 + 0.0024 \times \text{g} \\
\text{NFE} &= 0.0625 \times \text{ME} (\text{MJ}) \\
\end{align*}
\]

Fat corrected milk (4% FCM) was calculated according to Gaines (1928):

\[
\text{FCM (kg/d)} = \left[ \left( \% \text{ milk fat} \times 0.15 \right) + 0.4 \right] \times \text{milk yield} (\text{kg/d})
\]

P balance was calculated with the following equation:

\[
\text{P Balance (g/d)} = \text{P intake (g/d)} - \text{faecal P (g/d)} - \text{urea-P (g/d)} - \text{dietary P (g/d)}
\]

The native phytase activity for the corn silage is calculated according to tabulated values by Eckhout and Depape (1994). This results in a phytase activity of 12 FTU/kg DM. Daily duodenal DM flow (DMF) was calculated as follows:

\[
\text{DMF (kg/d)} = \frac{\text{Chromium application (mg/d)}}{\text{Duodenal chromium concentration (mg/g DM)}}
\]

The daily duodenal flows of OM and nutrients were estimated by multiplication of their respective concentrations in duodenal digesta with DMF. The utilisable CP (uCP) at the duodenum was estimated following Lebzien and Voigt (1999):

\[
\text{uCP (g/d)} = \text{CP-flow at the duodenum (g/d)} - \text{NH3-N} \times 0.25 \times \text{endogenous CP (g/d)}
\]

The endogenous CP (EP) was estimated following Brandt and Rohr (1981) using DMF at the duodenum:

\[
\text{EP (g/d)} = \left( 3.6 \times \text{kg DMF} \right) \times (6.25)
\]

The ruminal nitrogen balance (RNB), ruminally undegraded feed CP (RUP), ruminally degraded CP (RDP) and ruminally fermented OM (FOM) were calculated with the following equations:

\[
\begin{align*}
\text{RNB (g/d)} &= \text{CP-intake (g/d)} - \text{uCP (g/d)} \\
\text{RUP (g/d)} &= \text{CP} - \text{microbial N (g/d)} - \text{EP (g/d)} \\
\text{RDP (g/d)} &= \left( \text{CP-intake (g/d)} - \text{uCP (g/d)} \right) \\
\text{FOM (kg/d)} &= \text{OM intake (kg/d)} - \text{duodenal OM flow (kg/d)} - \text{omicrobial OM (kg/d)}
\end{align*}
\]

The microbial OM was calculated according to Schaft et al., (1983):

\[
\text{OM (kg/d)} = 11.8 \times \text{microbial N (kg/d)}
\]

The total tract digestibility was calculated with the following equation:

\[
\text{Total tract digestibility (g/d)} = \left( \% \text{ nutrient intake (g/d)} - \text{nutrient in faeces (g/d)} \right) \times 100
\]

The ME of the diets was calculated using the results from the sampling period and turned out to be 10.2±0.2, 10.4±0.2 and 10.1±0.2 MJ/kg DM for group LP, HP and LP+PHY. The NEL of the diets was 6.1±0.1, 5.9±0.2 and 5.9±0.2 MJ/kg DM for group HP, LP and LP+PHY.
Statistical analyses

The statistical analysis was carried out with the SAS-software package Version 9.1.3 (SAS, 2004). The procedure MIXED was used to analyse the data of intake, P concentration in milk, duodenal chyme, and faeces as well as rumen and duodenal variables. For repeated measures in ruminal fluid (pH-value, NH3-N and VFA) an autoregressive covariance structure was modelled using sampling time relative to feeding as the repeated effect. The models contained the treatment group as a fixed factor and the fact that each cow was used in several periods for different treatments was considered using a random statement for the individual animal variance. Variances were evaluated with the restricted maximum likelihood method and degrees of freedom were calculated according to the Kenward-Roger method. The pdiff option was used to determine significant effects between the least square means and Tukey-Kramer test was applied for post-hoc analysis. The results of the trial are presented as least squares (LS) means±standard error. Effects are graded as significant with P<0.05, a trend was considered if P<0.10 and P=0.05.

Results

The intended P concentrations in the diets of groups HP, LP and LP+PHY as well as the desired difference of 20% between the groups with or without P-supplementation were achieved. The average milk yield of 20.6 kg/d across all groups is required according to the GfE recommendations (2001) of 3.3 g P/kg DM in the diet. This content is equal to the analysed content in the HP diet. In LP and LP+PHY groups the dietary P content was reduced by 25.6 and 24.7%, respectively. The feed ingredients including corn silage, corn, wheat gluten and dried sugar beet pulp contributed to this comparatively low dietary P concentration as compared with other diets commonly used for dairy cows. The P concentration of the corn silage was on average 2.74 g/kg DM and the mean P concentration of the unsupplemented concentrates was 1.92 g/kg DM. There were no treatment effects on daily nutrient intakes (Table 2). The corn silage intake of the cows during the sampling period amounted on average to 9.8 kg DM/d. The mean DM intake from concentrate was 3.84±0.02, 3.85±0.03 and 3.83±0.03 kg/d in groups HP, LP and LP+PHY, respectively. The mean refusal weights were 0.05±0.2, 0.36±0.2 and 0.46±0.2 kg DM/d (P=0.436) for groups

| Table 2. Nutrient intakes by the fistulated cows during the sampling period (least squares means±standard error). |
|-------------------------------------------------|
| **Animals/group** | **Intakes, kg/d** | **CP** | **OM** | **EE** | **N** | **ADF** | **NDF** |
|-------------------|------------------|--------|--------|--------|-------|---------|---------|
|                   | HP               | LP     | LP+PHY | HP     | LP    | HP      | LP      |
| DM                | 13.6±0.15        | 13.3±0.18 | 13.6±0.18 | 0.414  |
| OM                | 12.7±0.13        | 12.9±0.15 | 12.8±0.15 | 0.709  |
| CP                | 1.8±0.17         | 1.8±0.20 | 1.8±0.20 | 0.602  |
| EE                | 0.4±0.01         | 0.4±0.01 | 0.4±0.01 | 0.335  |
| N                 | 0.3±0.003        | 0.3±0.003 | 0.3±0.003 | 0.602  |
| ADF               | 3.0±0.05         | 3.0±0.06 | 3.1±0.06 | 0.337  |
| NDF               | 6.0±0.09         | 5.8±0.11 | 6.1±0.11 | 0.318  |

P high phosphorus (diet added with dicalcium phosphate); LP, low phosphorus (diet without phosphorus and phytase supplementation); PHY, phytase (diet without phosphorus-supplementation, but added with an exogenous phytase); DM, dry matter; OM, organic matter; CP, crude protein; EE, ether extract; N, nitrogen; ADF, acid detergent fibre; NDF, neutral detergent fibre.

| Table 3. Effects of supplemental phosphorus and phytase on rumen fermentation parameters in dairy cows (least squares means±standard error). |
|-------------------------------------------------|
| **Animals/group** | **pH** | **NH3-N, mg/100 g** | **Total VFA, mmol/L** | **Acetic acid, mol %** | **Propionic acid, mol %** | **Butyric acid, mol %** | **Acetic/propanic acid** |
|-------------------|--------|---------------------|-----------------------|------------------------|--------------------------|-----------------------|------------------------|
|                   | HP     | LP               | LP+PHY               | HP                     | LP                       | LP+PHY                | HP                     |
|                   | 6.7±0.1 | 6.5±0.1          | 6.7±0.1               | 0.242                  | 13.1±2.4                 | 13.6±2.2              | 15.3±2.6               | 0.289                  |
|                   | 85.2±3.8 | 76.9±5.0         | 75.2±4.0              | 0.149                  | 59.4±1.1                 | 60.0±1.4              | 61.3±1.2               | 0.439                  |
|                   | 11.9±0.5 | 12.1±0.6         | 11.5±0.5              | 0.732                  | 27.7±1.1                 | 26.9±1.4              | 26.4±1.1               | 0.595                  |
|                   | 2.4±0.01 | 2.3±0.01         | 2.4±0.01              | 0.428                  | 52.9±1.2               | 53.2±1.54             | 53.5±1.36              | 0.389                  |
|                   | 3.0±0.12 | 2.9±0.16         | 3.2±0.13              | 0.392                  | 51.2±2.0                 | 50.1±3.0              | 54.3±3.0               | 0.561                  |
|                   | 1.7±0.06 | 1.8±0.09         | 1.8±0.07              | 0.361                  | 56.4±2.0                 | 55.9±3.0              | 59.5±3.0               | 0.670                  |
|                   | 7.5±0.15 | 7.4±0.20         | 7.5±0.18              | 0.854                  | 59.3±0.77                 | 58.5±1.02             | 58.3±0.92              | 0.635                  |
|                   | 65.9±2.06 | 58.9±2.40        | 55.1±2.13              | 0.001                  | 145.5±6.79               | 172.5±8.96             | 163.8±8.02              | 0.025                  |

P high phosphorus (diet added with dicalcium phosphate); LP, low phosphorus (diet without phosphorus and phytase supplementation); PHY, phytase (diet without phosphorus-supplementation, but added with an exogenous phytase); VFA, volatile fatty acids.

| Table 4. Effects of supplemental phosphorus and phytase on nutrient and phosphorus flows at the duodenum and amount of fermented organic matter in the rumen (least squares means±standard error). |
|-------------------------------------------------|
| **Animals/group** | **OM** | **NDF** | **ADF** | **FOM** | **P** |
|-------------------|--------|--------|--------|--------|------|
|                   | HP     | LP     | HP     | LP     | HP   |
|                   | OM/kd  | 6.7±0.12 | 6.8±0.17 | 6.8±0.15 | 0.810 |
|                   | % of intake | 52.9±1.12 | 53.2±1.54 | 53.5±1.36 | 0.952 |
|                   | NDF/kd | 3.0±0.12 | 2.9±0.16 | 3.2±0.13 | 0.389 |
|                   | % of intake | 51.2±2.0 | 50.1±3.0 | 54.3±3.0 | 0.561 |
|                   | ADF/kd | 1.7±0.06 | 1.8±0.09 | 1.8±0.07 | 0.361 |
|                   | % of intake | 56.4±2.0 | 55.9±3.0 | 59.5±3.0 | 0.670 |
|                   | FOM/kd | 7.5±0.15 | 7.4±0.20 | 7.5±0.18 | 0.854 |
|                   | % of intake | 59.3±0.77 | 58.5±1.02 | 58.3±0.92 | 0.635 |
|                   | P/g/d  | 65.9±2.06 | 58.9±2.40 | 55.1±2.13 | 0.001 |
|                   | % of intake | 145.5±6.79 | 172.5±8.96 | 163.8±8.02 | 0.025 |

P high phosphorus (diet added with dicalcium phosphate); LP, low phosphorus (diet without phosphorus and phytase supplementation); PHY, phytase (diet without phosphorus-supplementation, but added with an exogenous phytase); OM, organic matter; NDF, neutral detergent fibre; ADF, acid detergent fibre; FOM, fermented organic matter; P, phosphorus. *Different letters in the same row show significant differences (P<0.05).
HP, LP and LP+PHY.

Treatments did not affect rumen pH and ammonia-N concentration in rumen fluid (Table 3). The concentration of total VFA in rumen fluid was unchanged. No effects for molar percentage of acetic acid, propionic acid and butyric acid were observed among the three treatments. The acetic acid to propionic acid ratio was about 2.2 in the study and did not differ among the treatments. Treatments had no effect on the amount of FOM and the portion of OM intake fermented in the rumen. The ADF and NDF flow and its portion of intake showed no differences. The P-flow at the duodenum was higher in the HP-group (65 g/d) compared to the LP (58.9 g/d) and LP+PHY (55.1 g/d) group (P=0.001) (Table 4). The supplementation of feed with P and phytase had no effects on N flow at the duodenum, the rumen degradable and undegradable protein and the microbial protein synthesis (Table 5). There were no differences among the treatments for OM, CP and EE in faeces. The mean P concentration in urine was 0.05±0.04 g/kg DM. There were no differences among the treatments in the P concentration of urine and faeces (Table 6). Group LP showed a trend for a higher N concentration in urine (P=0.099). The treatments had no effect on total tract digestibility of OM, EE, NDF and ADF (Table 7). Milk yield amounted to 20.6±0.2 kg/d on average. No differences were observed in milk yield and milk composition among different treatments (Table 7). The mean P concentration in milk was 0.90 g P/kg milk and did not differ among the groups. The SCC showed no differences among the groups as well.

As intended by the experimental design, the P intake differed among the groups. Group LP and LP+PHY had nearly the same intake (33.5 vs 34.1 g P/d), group HP had a higher intake (45.3 g P/d) (P=0.0001). The P-excretion with faeces tended to be higher in group HP than in either other group (P=0.057) (Table 8). There was no influence of treatment on the secretion of P with milk during the sampling period. Urinary P-excretion showed a higher value in the HP-group (P=0.014). Group HP is the only group which showed a positive P-balance and differed compared to the LP and LP+PHY groups (P=0.01). However, there was no influence of treatment on the apparent total tract digestibility of P which averaged 47.5%.

### Discussion

Maenz (2001) investigated the occurrence of phytic acid in plants and found that cereals and grain legumes that are commonly used as feed ingredients have phytate levels, approximating 0.25% of DM. The InsP6 concentration in the concentrates was 0.57 g/kg DM in the LP+PHY group and on average 0.46 g/kg DM in the LP and LP+PHY group. Brask-Pedersen et al. (2013) found that an increase about four supplementation levels of exogenous P-phytase increased the ruminal degradation of InsP6. This degradation of InsP6 occurred mainly in the rumen and decreased InsP6 content in the duodenal chyme samples in animals fed phytase. This indicates that the supply of phytase increases the ruminal phytase activity. The phytase concentration in the diets of the current study are similar to the highly supple-

| Experimental diets | P |
|-------------------|---|
| HP                | 7 | 5 | 5 |
| LP                | 228±10 | 223±12 | 219±11 |
| LP+PHY            | 216±8.9 | 212±10.9 | 210±10.1 |

| Microbial CP |
|-------------|
| g/d         |
| 821±36.6    | 802±44.8 | 799±41.5 | 0.847 |
| 109±6.2     | 105±7.6  | 106±7.1  | 0.985 |
| 5.8±0.33    | 6.2±0.39 | 5.9±0.36 | 0.538 |
| 0.51±0.03   | 0.48±0.03 | 0.40±0.03 | 0.644 |

| Rumen undegradable protein |
|-----------------------------|
| g/d                         |
| 355±18.0                    | 339±21.1 | 328±20.0 | 0.400 |
| 20±0.97                     | 19±1.10  | 18±1.10  | 0.441 |
| 1429±20.6                   | 1434±27.2 | 1460±24.4 | 0.609 |
| 0.7±0.10                    | 0.8±0.14 | 0.6±0.12 | 0.421 |
| 1177±51.7                   | 1142±62.5 | 1127±58.3 | 0.679 |

Table 5. Effects of supplemental phosphorus and phytase on nitrogen flow at the duodenum as well as microbial protein synthesis and feed protein degradation in the rumen (least squares means±standard error).

| Experimental diets | P |
|-------------------|---|
| HP                | 7 | 5 | 5 |
| LP                | 899±4.0 | 888±4.8 | 901±4.8 | 0.097 |
| LP+PHY            | 132±2.4 | 130±2.8 | 135±2.8 | 0.280 |
| EE                | 33±2.2 | 34±2.6 | 33±2.6 | 0.940 |
| P                 | 4.9±0.32 | 4.3±0.36 | 4.5±0.36 | 0.257 |

| Urine |
|-------|
| g/kg DM |
| 0.10±0.03 | 0.02±0.03 | 0.04±0.03 | 0.180 |
| 5.58±1.09 | 6.60±1.18 | 5.36±1.19 | 0.099 |
| 21.3±5.9  | 21.5±5.8  | 20.9±5.8  | 0.497 |

Table 6. Effects of supplemental phosphorus and phytase on nutrient and phosphorus concentration of faeces, phosphorus- and nitrogen-concentration of urine in dairy cows as well as apparent total tract digestibility (least squares means±standard error).

**Phytase in dairy cows**

**Phytase in dairy cows**

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mented group of the study by Brask-Pedersen et al. (2013). However, the results of the actual study for P-balance data with no differences between the LP and LP+PHY group indicate that InsP₆ degradation and absorption of released P in the duodenum as a result of ruminal phytase activity is not influenced by phytase supplementation. Dvorakova (1998) determined that the optimum of pH is 2.5 or 5.5 for Aspergillus niger phytase, while Brask-Pedersen et al. (2013) mentioned that the optimum pH for the efficiency of the phytase used in their study was 5.0 to 5.5. The pH-value of the rumen of the cows in the current study is on average 6.6. The time per day the ruminal pH spent below 5.6 was determined in a study by Lohöter et al. (2013) and was on average 291 min/d or 20% of the whole day. Times when the ruminal pH coincided with the pH-optimum of the phytase are the exception rather than the rule and maybe a possible reason for the absence of phytase effects on the P-balance in the current study. Post ruminal phytate degradation was not observed because of the unchanged apparent total tract digestibility.

Effects of dietary P deficiency with an insufficient P-supply to the rumen microbes on the microbial metabolism are reduced feed intake, OM digestibility and efficiency of microbial protein synthesis (Breves and Schröder, 1991; Kincad and Rodehutsdorc, 2005). In the present study the effects on rumen fermentation characteristics were only marginal. Parameters of microbial protein synthesis (Table 5) and OM digestibility (Table 6) were not influenced in groups with reduced P-supply. This suggested that P recycling via saliva was sufficient to supply the requirements of the microbes, even though the groups experienced a P deficiency in the diet. The duodenal P-flow markedly higher than 100 percent of intake in all groups confirmed this suggestion. The unchanged ammonia concentrations in the rumen fluid indicated no effect of P or phytase supplementation on protein degradation in the rumen.

Values for P in the milk of lactating Holstein cows determined over the complete lactation range are between 0.85-0.94 g P/kg milk (Brintrup et al., 1993; Valk et al., 2002; Wu et al., 2001). In the present trial the values amounted to 0.90 g P/kg milk. They are similar to the mean concentration of 0.9 g P/kg milk given by Pfeffer et al. (2005) and to the mean concentration of 0.89 g P/kg milk given by Klop et al. (2013). The present results confirm the statement of Pfeffer et al. (2005) that the P-intake has no influence on the P-excretion with milk. In contrast to the P-excretion with milk, the P-excretion with faeces tended to be higher in the HP-group (P=0.057) and faecal P-excretion was unaffected by the exogenous phytase fed to cows. The HP-group excreted 22 g P/d, while the LP-group excreted 19 g and the LP+PHY-group excreted 18 g P/d. This results in a difference of 14%, resp. 18%, compared to the HP group. The present results confirm the statement of Pfeffer et al. (2005) and other authors who found a direct correspondence between P-intake and P-excretion in dairy cows (Knowlton and Herbein, 2002; Knowlton et al., 2004; Wu et al., 2001). Hill et al. (2008) observed that total P excreted with faeces is not very sensitive to supplemented phytase and is comparable to the results of the present study. In contrast, dietary P-supplementation has a positive effect on the P-balance in the current study. The P-balance for group LP and LP+PHY was negative, while it was positive for the HP group. The HP-group showed a higher P-balance (P=0.010) compared with both other groups. In the current study, a mineral P-intake according to the GIE recommendations (2001) enabled the cows to retain more P. Valk et al. (2002) found comparable results for lactating dairy cows. The P-balance of cows calculated by Hill et al. (2008) became negative at a similar dietary P content, which is slightly less

| Experimental diets | P |
|--------------------|---|
| Animals/group      | 7 | 5 | 5 |
| Milk yield, kg/d   | 20.5±1.6 | 20.8±1.9 | 20.5±1.7 | 0.984 |
| FCM, kg/d          | 19.4±1.31 | 19.3±1.60 | 20.0±1.45 | 0.934 |
| Milk composition, %|   |   |   |   |
| Fat                | 3.8±0.23 | 3.6±0.26 | 3.7±0.25 | 0.768 |
| Protein            | 2.7±0.07 | 2.7±0.09 | 2.8±0.07 | 0.351 |
| Lactose            | 4.8±0.08 | 4.8±0.09 | 4.8±0.09 | 0.982 |
| Milk yield, g/d    | 750±51.7 | 746±51.1 | 774±55.8 | 0.931 |
| Protein            | 556±35.9 | 570±42.1 | 559±38.4 | 0.949 |
| Lactose            | 978±80.1 | 975±95.6 | 988±86.5 | 0.993 |
| Urea, ppm          | 115±19.0 | 132±21.6 | 117±20.0 | 0.696 |

Table 7. Effects of supplemental phosphorus and phytase on milk production and composition in dairy cows (least squares means±standard error).

| Experimental diets | P |
|--------------------|---|
| Animals/group      | 7 | 5 | 5 |
| P , g/d            | 45±0.7± | 34±0.8± | 34±0.8± | <0.001 |
| Excretion with faeces  | 22±1.0 | | | |
| P , g/d            | 2.4±0.52± | 0.2±0.62± | 0.1±0.62± | 0.041 |
| Excretion with urine | 19.5±1.5 | 19±1.8 | 19±1.8 | 0.984 |
| P , g/d            | 2.6±1.25± | -3.2±1.48± | -3.0±1.48± | 0.010 |
| Apparent total tract digestibility | 52±2.5 | 44±3.1 | 47±3.1 | 0.126 |

Table 8. Mean phosphorus-intake and phosphorus secretion with milk, phosphorus secretion with faeces, urine as well as phosphorus-balance of the dairy cows during the sampling period (least squares means±standard error).

HP: high phosphorus (diet added with dicalcium phosphate); LP: low phosphorus (diet without phosphorus and phytase supplementation); PHY, phytase (diet without phosphorus-supplementation, but added with an exogenous phytase); FCM, fat corrected milk.
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