Effects of Dietary Cellulose on the Basal Endogenous Loss of Phosphorus in Growing Pigs

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ABSTRACT: An experiment was conducted to determine the effect of cellulose concentration in diets containing no phosphorus (P) on the basal endogenous loss (BEL) of P in growing pigs. Twelve barrows (an initial mean body weight = 49.6±3.2 kg) were individually housed in metabolism crates. Pigs were allotted to 4 experimental diets according to a cross-over design with 12 animals and 2 periods. Four P-free diets were mainly based on corn starch, sucrose, and gelatin, and were formulated to contain 0%, 4%, 8%, or 12% cellulose. Each period consisted of a 5-d adaptation and a 5-d collection period. The marker-to-marker method was used for fecal collection. The feed intake (p<0.05, linear and quadratic) and dry feces output (p<0.01, linear and quadratic) were increased with increasing dietary cellulose concentration. However, P concentration in the feces was decreased (p<0.01, linear and quadratic) with increasing dietary cellulose concentration. There was no significant difference in total P output and the BEL of P as mg per kg DMI (ranging from 157 to 214 mg/kg of dry matter intake) among experimental diets. However, values for the apparent total tract digestibility of energy, dry matter, organic matter, crude protein, and calcium were linearly decreased (p<0.01) with increasing cellulose concentration in the diet. In conclusion, dietary cellulose affected the amount of feces and digestibility of energy and nutrients, but did not affect the endogenous loss of P. (Key Words: Digestibility, Endogenous Excretion, Fiber, Phosphorus, Swine)

INTRODUCTION

The standardized total tract digestibility (STTD) is more accurate than the apparent total tract digestibility (ATTD) for determination of phosphorus (P) availability (Almeida and Stein, 2011; Son et al., 2013). Moreover, the value for STTD of P is considered more additive than the ATTD in mixed diets (Almeida and Stein, 2010), which enables an accurate formulation swine diets. The ATTD of P is corrected by the basal endogenous loss (BEL) of P from pigs fed a P-free diet to calculate the STTD of P (Kim et al., 2012).

In case of nitrogen, an increase of dietary fiber content leads to increase ileal nitrogen losses (Schulze et al., 1994; Gabert et al., 2001). There was a hypothesis that high dietary fiber content may increase endogenous losses of P (Petersen and Stein, 2006; Son et al., 2013). If the value for the BEL of P estimated from the pig fed a P-free diet is affected by the fiber concentration in the diet, the STTD of P may be largely influenced by dietary fiber. However, the effect of dietary fiber concentration on the endogenous loss of P has not been documented. Therefore, the objective of this study was to determine the effects of fiber concentration in the P-free diets on the BEL of P in growing pigs.

MATERIALS AND METHODS

Animal care
The experimental procedure was approved by the Institutional Animal Care and Use Committee at Konkuk University.

Animal, diet, and feeding
Twelve barrows with an initial mean body weight (BW) of 49.6 kg (standard deviation = 3.2) were used to determine the effects of dietary fiber concentration in the P-free diet on the BEL of P. Pigs were individually housed in
metabolic creates equipped with a feeder and a nipple drinker.

Four P-free experimental diets were prepared (Tables 1 and 2). The experimental diets were mainly based on corn starch, sucrose, and gelatin and were formulated to contain 0%, 4%, 8%, or 12% cellulose (Comprecel, Mingtai Chemical Co., LTD, Bah-Der, Taiwan). The inclusion rate of cellulose was decided based on the values in the literature (Almeida and Stein, 2011; Kim et al., 2012; Son et al., 2013). Vitamins and minerals were adequate to meet or exceed requirement estimates of NRC (1998).

The pigs were allotted to 4 dietary treatments in a cross-over design with 12 animals and 2 periods. The amount of daily feed allowance per pig was calculated as approximately 2.5 times the estimated energy requirement for maintenance (i.e., 106 kcal of metabolizable energy [ME]/kg of BW0.72; NRC, 1998). The daily feed allowance divided into 2 equal meals, and fed to pigs at 0800 and 1600. At the beginning of each period, the BW of pigs was measured to determine the daily feed allowance. Water was available at all times. Considering the potential problems in pigs fed the P deficient diet for a long time, a commercial corn-soybean meal-based diet was fed to the pigs for 7 d between the 2 periods.

Sample collection

Each experimental period consisted of a 5-d adaptation period and 5-d collection period. A total amount of feces was collected according to a marker-to-marker procedure (Kong and Adeola, 2014). Chromium oxide was added at 0.5% to the morning meal as an indigestible marker on d 6 and 11 in each period. Fecal collection was started when the color of marker begin to appear in the feces, and ended when the color appeared again. Collected fecal samples were immediately stored at –20°C in the freezer until subsequent chemical analyses.

Chemical analysis

The total amount of feces samples was dried in a forced-air drying oven at 55°C and then ground for analyses. Diet and feces samples were dried in the forced-air drying oven at 135°C for 2 h to analyze dry matter (DM; method 930.15; AOAC, 2005). Diet and feces samples were analyzed for gross energy (Parr 1261 bomb calorimeter; Parr Instruments Co., Moline, IL, USA), crude protein (CP; method 990.03; AOAC, 2005), ether extract (EE; method 920.39; AOAC, 2005), and ash (method 942.05; AOAC, 2005). Calcium (Ca) in diet and feces samples was analyzed (method 978.02; AOAC, 2005) using an atomic absorption spectrophotometer (Perkin Elmer 3300, Perkin Elmer, Akron, OH, USA). Diet samples were also analyzed for crude fiber (method 978.10; AOAC 2005), neutral detergent fiber (NDF; Goering and van Soest, 1970), and acid detergent fiber (method 973.18; AOAC, 2005). Phosphorus in feces samples was analyzed (method 946.06; AOAC, 2005) using a spectrophotometer (Optizen 2120UV, Mecasys, Daejeon, Korea).

Statistical analysis

Data were analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC, USA). Two outliers (difference from median >2x interquartile range) were identified in 2 experimental diets, and were removed from the final data set. The model included dietary treatment as a fixed factor.

Table 1. Ingredient composition of experimental diets, as-fed basis

| Ingredient (%) | Dietary cellulose concentration (%) |
|----------------|--------------------------------------|
|                | 0  | 4  | 8  | 12 |
| Corn starch    | 55.19 | 51.19 | 47.19 | 43.19 |
| Sucrose        | 20.00 | 20.00 | 20.00 | 20.00 |
| Gelatin        | 17.00 | 17.00 | 17.00 | 17.00 |
| Soybean oil    | 4.70 | 4.70 | 4.70 | 4.70 |
| Cellulose1     | -   | 4.00 | 8.00 | 12.00 |
| DL-methionine  | 0.30 | 0.30 | 0.30 | 0.30 |
| L-threonine    | 0.10 | 0.10 | 0.10 | 0.10 |
| L-tryptophan   | 0.11 | 0.11 | 0.11 | 0.11 |
| L-histidine    | 0.10 | 0.10 | 0.10 | 0.10 |
| L-isoleucine   | 0.10 | 0.10 | 0.10 | 0.10 |
| Ground limestone | 1.00 | 1.00 | 1.00 | 1.00 |
| Potassium carbonate | 0.40 | 0.40 | 0.40 | 0.40 |
| Magnesium oxide | 0.10 | 0.10 | 0.10 | 0.10 |
| Salt           | 0.40 | 0.40 | 0.40 | 0.40 |
| Vitamin-mineral premix2 | 0.50 | 0.50 | 0.50 | 0.50 |

1 Comprecel (Mingtai Chemical Co., LTD, Bah-Der, Taiwan).
2 Provided the following quantities per kg of complete diet: vitamin A, 25,000 IU; vitamin Ds, 4,000 IU; vitamin E, 50 IU; vitamin K, 5.0 mg; thiamin, 4.9 mg; riboflavin, 10.0 mg; pyridoxine, 4.9 mg; vitamin B6, 0.06 mg; pantothenic acid, 37.5 mg; folic acid, 1.10 mg; niacin, 62 mg; biotin, 0.06 mg; Cu, 25 mg as copper sulfate; Fe, 268 mg as iron sulfate; I, 5.0 mg as potassium iodate; Mn, 125 kg as manganese sulfate; Se, 0.38 mg as sodium selenite; Zn, 313 mg as zinc oxide; butylated hydroxytoluene, 50 mg.

Table 2. Chemical composition of experimental diets1, as-fed basis

| Item                          | Dietary cellulose concentration (%) |
|-------------------------------|--------------------------------------|
|                               | 0   | 4   | 8   | 12  |
| Dry matter (%)                | 91.0| 91.2 | 91.8| 92.1|
| Gross energy (kcal/kg)        | 4,091| 4,086| 4,098| 4,090|
| Metabolizable energy2 (kcal/kg) | 3,717| 3,558| 3,398| 3,239|
| Crude protein (%)             | 18.6| 19.1| 18.5| 17.5|
| Ether extract (%)             | 4.31| 4.60| 4.84| 4.64|
| Ash (%)                       | 2.77| 3.64| 3.14| 3.74|
| Calcium (%)                   | 0.51| 0.55| 0.52| 0.50|
| Neutral detergent fiber (%)   | 3.55| 7.33| 10.1| 13.5|
| Acid detergent fiber (%)      | 0.76| 3.36| 4.83| 10.9|
1 Data are the mean of duplicate analyses of each diet
2 Values are calculated based on NRC (1998) for each ingredient.
fixed variable and replication, animal within replication, and period as random variables. Orthogonal polynomial contrasts were used to test linear and quadratic responses of treatment. Least squares means of each treatment were calculated. The experimental unit was a pig, and an alpha level of 0.05 was used to test the statistical significance.

**RESULTS**

There were no health problems observed in the pigs during the experimental period. Daily feed intake and dry matter intake (DMI) were linearly and quadratically increased (p<0.05) with increasing dietary cellulose concentration (Table 3). Daily total fecal output was linearly and quadratically increased (p<0.01) with increasing dietary cellulose concentration. The fecal output per kg DMI were also linearly and quadratically increased (p<0.05) with increasing dietary cellulose concentration. However, the P concentration in the feces was decreased (p<0.01) with increasing dietary cellulose concentration. There were no differences in the total P output and the BEL of P as mg per kg DMI among experimental diets.

The energy concentration in the feces was not different among experimental diets (Table 4). The DM concentration in the feces was linearly increased (p<0.05) with increasing dietary cellulose concentration. The organic matter (OM), CP, EE, and Ca concentrations in the feces were linearly and quadratically increased (p<0.01) with increasing dietary cellulose concentration. The ATTD of energy and CP were linearly decreased (p<0.01) with increasing dietary cellulose concentration. The ATTD of DM, OM, and Ca were linearly and quadratically decreased (p<0.05) with increasing dietary cellulose concentration. However, there was no difference

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**Table 4. Energy and nutrients concentrations in the feces and the apparent total tract digestibility (ATTD) of energy and nutrients in the phosphorus-free diets with 0% to 12% of cellulose fed to growing pigs, as-fed basis**

| Item          | Dietary cellulose concentration (%) | SEM      | p-value |
|---------------|-------------------------------------|----------|---------|
|               | 0        | 4        | 8        | 12       |          | Linear | Quadratic |
| Observation (n) | 5        | 6        | 6        | 5        |          |         |          |
| Concentration in feces (%) |          |          |          |          |          |         |          |
| Energy (kcal/kg) | 3.971    | 3.771    | 3.816    | 3.914    | 89       | 0.691   | 0.079    |
| Dry matter     | 94.1     | 95.5     | 96.0     | 96.2     | 1.2      | 0.030   | 0.375    |
| Organic matter | 61.2     | 81.2     | 86.7     | 89.2     | 1.0      | <0.001  | <0.001   |
| Crude protein  | 16.8     | 6.76     | 4.75     | 5.28     | 0.97     | <0.001  | <0.001   |
| Ether extract  | 3.73     | 1.02     | 0.66     | 0.70     | 0.15     | <0.001  | <0.001   |
| Calcium        | 7.53     | 3.74     | 2.52     | 1.92     | 0.37     | <0.001  | 0.001    |
| ATTD (%)       |          |          |          |          |          |         |          |
| Energy         | 98.6     | 94.9     | 91.9     | 89.7     | 0.3      | <0.001  | 0.073    |
| Dry matter     | 98.5     | 94.2     | 90.7     | 88.4     | 0.4      | <0.001  | 0.022    |
| Organic matter | 99.0     | 94.9     | 91.3     | 88.8     | 0.3      | <0.001  | 0.045    |
| Crude protein  | 98.6     | 98.0     | 97.7     | 96.7     | 0.3      | <0.001  | 0.360    |
| Ether extract  | 98.7     | 98.8     | 98.8     | 98.3     | 0.2      | 0.114   | 0.126    |
| Calcium        | 78.4     | 62.8     | 57.1     | 58.2     | 2.4      | <0.001  | 0.015    |

SEM, standard error of the means.
in the ATTD of EE among the experimental diets.

**DISCUSSION**

Cellulose has been often used as a fiber source in a P-free diet in previous experiments (Almeida and Stein, 2011; Kim et al., 2012; Son et al., 2013). The influence of dietary fiber on nutrient utilization (Ravindran et al., 1984; Wilfart et al., 2007) and endogenous nitrogen excretion (Schulze et al., 1994; Yin et al., 2000) has been documented. To the best of our knowledge, the present experiment first addresses the effects of graded concentrations of dietary cellulose on the BEL of P in pigs.

The differences of the NDF concentration among experimental diets were similar to the intended differences when the diets were formulated with graded concentrations of cellulose in the present work. The NDF concentration in the purified diet without cellulose was analyzed to be 3.55%, which may be due to the small quantities of NDF in corn starch, sucrose, and gelatin.

In this study, we tried to synchronize ME intake among the treatment groups and the daily feed allowance was calculated based on the BW and the ME concentration of each experimental diet. The daily feed allowance was increased with increasing dietary fiber concentration because calculated ME concentrations in the experimental diets decreased with increasing dietary fiber concentration (Table 2). Therefore, the differences in the feed intake were expected results.

Dietary fiber did not affect the total P output or the BEL of P. The fecal output was increased, but the P concentration in the feces was decreased with increasing dietary cellulose concentration. This subsequently caused insignificant differences of the P output and the BEL of P regardless of dietary cellulose concentration. The reason why we failed to find the effect of dietary cellulose on endogenous P excretion was likely associated with the structure of the cellulose source. The cellulose source used in this experiment was a microcrystalline cellulose product which was partially depolymerized cellulose from purified wood pulp and thus had perhaps a minimal physical influence on sloughing of intestinal cells. This theory is well supported by previous studies that measured ileal endogenous excretions of nitrogen by feeding various sources of fiber. Schulze et al. (1994) suggested that purified NDF induced an increased excretion of endogenous ileal nitrogen at least partially due to the intact fibrous structure of the purified NDF. However, purified wood cellulose in nitrogen-free diets did not influence the ileal amino acids or nitrogen excretion (Furuya and Kaji, 1992) which was similar to the present endogenous P results. Physical or chemical properties of dietary fiber may be a more critical factor for ileal endogenous nitrogen excretion than the concentration of dietary fiber (Leterme et al., 1996; Souffrant, 2001; Libao-Mercado et al., 2006; Park et al., 2013).

The decreased ATTD of energy, DM, and OM with increasing dietary fiber concentration in this experiment was likely due to the very low concentration of digestible carbohydrates in the cellulose which was added at the expense of corn starch. Another reason may be an increased passage rate with increasing dietary cellulose concentration. While the time for marker appearance in feces after feeding was 2 to 4 days in pigs fed 4%, 8%, or 12% cellulose diets, it was at least 7 days in pigs fed a 0% cellulose diet (data not shown). A fast passage rate of digesta due to high dietary fiber concentrations was associated with low digestibility of energy and nutrients (Ravindran et al., 1984), which coincided with the present results. Moreover, a slower passage rate resulted in a higher DM digestibility in pigs, which was independent of dietary fiber concentration (Kim et al., 2007).

The decreased nutrient digestibility with increasing dietary fiber concentration in the present study was consistent with data in the literature (Kass et al., 1980; Ravindran et al., 1984; Le Goff et al., 2002). Although the possible influence of feed intake on the nutrient digestibility should not be neglected, the extent of feed intake variation would not largely affect the nutrient digestibility (Moter and Stein, 2004). Another factor affecting the nutrient digestibility was the initial BW, but the relatively narrow range of initial BW (from 44.2 to 56.4 kg) would not be the reason for the different DM digestibility (Kim et al., 2007).

In contrast to energy, OM, and DM, the ATTD of EE was not affected by dietary cellulose concentration. Generally, extracted free-form oil sources are very easily digested and absorbed in the small intestine compared with intact-form oil sources (Kim et al., 2013). Soybean oil, a free-form source, mostly contributed to the dietary EE in this experiment. The time of digesta retention in the intestinal tract was likely sufficient for the efficient digestion and absorption of the free-form oil.

**CONCLUSION**

The ATTD of energy, DM, OM, and other nutrients decreases with increasing dietary microcrystalline cellulose concentration. However, the microcrystalline cellulose did not affect the endogenous loss of P. Further studies to test the effects of a crude type of cellulose and different sources of dietary fiber on the endogenous excretion of P are warranted in the future.

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