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# Effects of microbial phytase on apparent and standardized total tract digestibility of calcium in calcium supplements fed to growing pigs[1]

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**ABSTRACT:** An experiment was conducted to test the hypothesis that differences in the apparent total tract digestibility (ATTD) and standardized total tract digestibility (STTD) of Ca exist among Ca supplements and that inclusion of microbial phytase increases the ATTD and STTD of Ca. One hundred and four growing barrows (average initial BW of 17.73 ± 2.53 kg) were allotted to a randomized complete block design with 13 dietary treatments and 8 pigs per treatment. A basal diet containing corn, cornstarch, potato protein isolate, soybean oil, calcium carbonate, monosodium phosphate, vitamins, and minerals was formulated. Five additional diets were formulated by adding monocalcium phosphate (MCP), dicalcium phosphate (DCP), calcium carbonate, *Lithothamnium calcareum* Ca, or a high-Ca sugar beet co-product to the basal diet at the expense of cornstarch. Six additional diets that were similar to the previous 6 diets with the exception that they also contained 500 units per kilogram of microbial phytase were also formulated. A Ca-free diet was used to determine basal endogenous losses of Ca. Feces were collected using the marker-to-marker approach. Results indicated that regardless of inclusion of microbial phytase, MCP had the greatest ($P < 0.05$) ATTD and STTD of Ca. The ATTD and STTD of Ca in DCP were greater ($P < 0.05$) than in calcium carbonate, *L. calcareum* Ca, or in the sugar beet co-product, but no differences were observed among the ATTD and STTD of Ca in calcium carbonate, *L. calcareum* Ca, or sugar beet co-product. Inclusion of microbial phytase increased ($P < 0.05$) the ATTD and STTD of Ca in the diets, but this was not the case in the Ca supplements. Regardless of inclusion of microbial phytase, the ATTD of P was greater ($P < 0.05$) in pigs fed basal, MCP, or DCP diets than in pigs fed calcium carbonate, *L. calcareum* Ca, or the sugar beet co-product, but pigs fed calcium carbonate diets had greater ($P < 0.05$) ATTD of P than pigs fed *L. calcareum* Ca or the sugar beet co-product. Regardless of Ca source, inclusion of microbial phytase increased ($P < 0.001$) the ATTD of P. In conclusion, MCP has the greatest ATTD and STTD of Ca among the calcium supplements used in this experiment, followed by DCP. Basal, MCP, and DCP diets had greater ATTD of P than the other diets, and inclusion of microbial phytase increased the ATTD and STTD of Ca and the ATTD of P in the diets.

**Key words:** apparent digestibility, calcium, calcium supplements, phytase, pigs, standardized digestibility

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## INTRODUCTION

Most Ca in diets fed to pigs is supplemented as inorganic Ca because of the low concentration of Ca in most plant-based feed ingredients. In a typical corn–soybean meal diet for a 40-kg pig, the Ca contribution from corn and soybean meal is around 1 g per kg of diet, whereas approximately 5 g of Ca per kg of diet is supplied by limestone and calcium phosphates (NRC, 1998). Apparent total tract digestibility (**ATTD**) values for Ca in some ingredients have been reported (Bohlke et al., 2005; Stein et al., 2011; González-Vega et al., 2013), but for most commonly used Ca sources, digestibility values are not available. It has been demonstrated that values for ATTD of Ca may be influenced by the concentration of Ca in the diet, which is a result of the endogenous loss of Ca from the intestinal tract of pigs (González-

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Vega et al., 2013). Therefore, it is likely that values for standardized total tract digestibility (**STTD**) of Ca are more accurate to use in diet formulations than values for ATTD because STTD values are additive in mixed diets (NRC, 2012). However, for most feed ingredients, values for the STTD of Ca are not available, but the relative bioavailability of Ca may vary among Ca supplements (Ross et al., 1984). It is, therefore, expected that STTD of Ca also differs among Ca sources.

Inclusion of microbial phytase in swine diets often increases the digestibility of Ca and P (Brady et al., 2002; Liao et al., 2006; Poulsen et al., 2010), but effects of phytase on the STTD of Ca in individual ingredients have not been reported. Therefore, the objectives of this experiment were to test the hypothesis that 1) differences in the ATTD and STTD of Ca exist among Ca supplements and 2) that inclusion of microbial phytase in the diets increases the ATTD and STTD of Ca. If these hypotheses are confirmed, it will be concluded that diets fed to pigs are most accurately formulated if values for the STTD of Ca in all feed ingredients are used.

## MATERIALS AND METHODS

The Institutional Animal Care and Use Committee at the University of Illinois reviewed and approved the protocol for the experiment. Pigs used in the experiment were the offspring of G-Performer boars and Fertilis 25 females (Genetiporc, Alexandria, MN).

### *Animals and Housing*

One hundred and four growing barrows with an average initial BW of 17.73 ± 2.53 kg were randomly allotted to 13 diets with 8 replicate pigs per treatment using the Experimental Animal Allotment Program (Kim and Lindemann, 2007). The weight of the pigs was used as the block for the allotment. Pigs were housed individually in metabolism crates that were equipped with a feeder, a nipple drinker, a slatted floor, and a screen floor for total fecal collection. This experiment was conducted in 5 blocks, 3 blocks with 2 replicate pigs per diet and 2 blocks with 1 pig per diet.

### *Diets and Feeding*

The sources of Ca that were used in this experiment were monocalcium phosphate (**MCP**), dicalcium phosphate (**DCP**), calcium carbonate, *Lithothamnium calcareum* Ca (Vistacal; AB Vista Feed Ingredients, Marlborough, UK), and a sugar beet co-product (Limex; British Sugar PLC, Peterborough, UK; Table 1). Monocalcium phosphate and DCP were obtained from PCS Sales (Northbrook, IL). Calcium carbonate was obtained from ILC Resources (Urbandale, IA). *Lithothamnium calcareum* Ca is a source of Ca produced by calcified seaweeds, *Lithothamnium calcareum*, and was obtained from Celtic Sea Minerals (Currabinny, Co. Cork, Ireland). The sugar beet co-product was obtained during sugar juice purification and is a source of Ca in the form of calcium dihydroxide precipitated with carbon dioxide (Associated British Foods Annual Report and Accounts, 2008).

A basal diet containing corn, cornstarch, potato protein isolate, soybean oil, calcium carbonate, monosodium phosphate (**MSP**), vitamins, and minerals was formulated (Table 2). This diet contained 0.33% Ca and 0.42% total P (Table 3). Two diets containing 0.70 and 0.79% Ca and 0.64 and 0.67% total P were formulated by adding MCP or DCP, respectively, to the basal diet and the inclusion of MSP was eliminated. Three additional diets that all contained between 0.75 and 0.86% Ca and between 0.40 and 0.42% total P were formulated by adding calcium carbonate, *L. calcareum* Ca, or the sugar beet co-product to the basal diet at the expense of cornstarch.

Six additional diets that were similar to the previous 6 diets, with the exception that they also contained 500 units per kg of microbial phytase (Quantum Blue, AB Vista Feed Ingredients, Marlborough, UK), were also formulated. Microbial phytase was included in these diets at the expense of cornstarch. A Ca-free diet that was used to measure basal endogenous losses of Ca was also formulated. This diet contained cornstarch, potato protein isolate, sucrose, soybean oil, Solka floc, MSP, crystalline AA, vitamins, and minerals.

Pigs were fed each diet for 13 d and the feed allotment was calculated as 3 times the daily maintenance energy requirement (i.e., 106 kcal of ME/kg $BW^{0.75}$; NRC, 1998). The allotments of feed were divided into 2 equal meals and provided at 0700 and 1700 h daily. Pigs had free access to water throughout the experiment. The initial 5 d was an adaptation period to the diets and fecal samples were collected quantitatively from d 6 to 11 using the marker-to-marker approach (Adeola, 2001). Indigo carmine was the indigestible marker added to the morning meal on d 6 to mark the beginning of fecal collection. Ferric oxide was the marker added to the morning meal on d 11 to mark the end of fecal collection. Fecal samples were stored at −20°C immediately after collection and samples were dried in a forced-air oven at 65°C and finely ground through a 1-mm screen in a Wiley mill (model 4; Thomas Scientific, Swedesboro, NJ) before laboratory analysis.

### *Sample Analysis*

Corn, potato protein isolate, MCP, DCP, calcium carbonate, *L. calcareum* Ca, the sugar beet co-product,

**Table 1.** Analyzed composition of ingredients, as-fed basis

| | Ingredient[1] | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Item | Corn | Potato protein isolate | MCP | DCP | Calcium carbonate | *L. calcareum* Ca | Sugar beet co-product | MSP |
| GE, kcal/kg | 3,925 | 5,252 | — | — | — | — | — | — |
| DM, % | 89.06 | 91.95 | 93.50 | 95.09 | 99.94 | 99.27 | 92.01 | 98.99 |
| Ash, % | 1.22 | 0.51 | 81.36 | 84.66 | 94.53 | 92.97 | 84.21 | 90.61 |
| CP, % | 8.12 | 81.97 | — | — | — | — | — | — |
| AEE,[2] % | 3.73 | 0.16 | — | — | — | — | — | — |
| ADF, % | 2.74 | 4.59 | — | — | — | — | — | — |
| NDF, % | 11.54 | 1.32 | — | — | — | — | — | — |
| Ca, % | 0.01 | 0.04 | 18.54 | 21.61 | 41.82 | 34.57 | 31.70 | 0.09 |
| P, % | 0.26 | 0.15 | 21.60 | 19.21 | 0.16 | 0.22 | 0.81 | 27.10 |
| Phytate, % | 0.75 | 0.34 | — | — | — | — | — | — |
| Phytate-bound P,[3] % | 0.16 | 0.10 | — | — | — | — | — | — |
| Non-phytate P,[4] % | 0.10 | 0.05 | — | — | — | — | — | — |

[1]MCP = monocalcium phosphate; DCP = dicalcium phosphate; MSP = monosodium phosphate. *L. calcareum* Ca is a source of Ca produced by *Lithothamnium calcareum* (Celtic Sea Minerals, Currabinny, Co. Cork, Ireland). The sugar beet co-product is a source of Ca in the form of calcium dihydroxide precipitated with carbon dioxide during sugar juice purification (British Sugar PLC, Peterborough, UK).

[2]AEE = acid-hydrolyzed ether extract.

[3]Phytate-bound P was calculated as 28.2% of phytate (Tran and Sauvant, 2004).

[4]Non-phytate P was calculated as the difference between total P and phytate-bound P.

**Table 2**. Ingredient composition of experimental diets without microbial phytase, as-fed basis

| | Diet[1] | | | | | | |
|---|---|---|---|---|---|---|---|
| Ingredient, % | Basal | MCP | DCP | Calcium carbonate | *L. calcareum* Ca | Sugar beet co-product | Ca-free |
| Corn | 77.00 | 77.00 | 77.00 | 77.00 | 77.00 | 77.00 | — |
| Cornstarch | 9.60 | 8.35 | 8.20 | 8.40 | 8.15 | 8.00 | 58.57 |
| Potato protein isolate | 8.00 | 8.00 | 8.00 | 8.00 | 8.00 | 8.00 | 10.00 |
| Soybean oil | 3.00 | 3.00 | 3.00 | 3.00 | 3.00 | 3.00 | 4.00 |
| Calcium carbonate | 0.80 | 0.80 | 0.80 | 2.00 | 0.80 | 0.80 | — |
| Monocalcium phosphate | — | 2.00 | — | — | — | — | — |
| Dicalcium phosphate | — | — | 2.15 | — | — | — | — |
| *L. calcareum* Ca[2] | — | — | — | — | 1.45 | — | — |
| Sugar beet co-product[3] | — | — | — | — | — | 1.60 | — |
| Monosodium phosphate | 0.75 | — | — | 0.75 | 0.75 | 0.75 | 0.95 |
| L-Lys HCl, 78% Lys | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 |
| DL-Met | — | — | — | — | — | — | 0.11 |
| L-Trp | — | — | — | — | — | — | 0.02 |
| Potassium carbonate | — | — | — | — | — | — | 0.40 |
| Magnesium oxide | — | — | — | — | — | — | 0.10 |
| Solka floc[4] | — | — | — | — | — | — | 5.00 |
| Sucrose | — | — | — | — | — | — | 20.00 |
| Sodium chloride | 0.40 | 0.40 | 0.40 | 0.40 | 0.40 | 0.40 | 0.40 |
| Vitamin mineral premix[5] | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 |
| Total | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |

[1]MCP = monocalcium phosphate; DCP = dicalcium phosphate; Six additional diets that were similar to the above diets, with the exception that 500 units per kilogram of microbial phytase (Quantum Blue, AB Vista Feed Ingredients, Marlborough, UK) was included in the diets at the expense of cornstarch were also formulated.

[2]*L. calcareum* Ca is a source of Ca produced by *Lithothamnium calcareum* (Celtic Sea Minerals, Currabinny, Co. Cork, Ireland).

[3]The sugar beet co-product is a source of Ca in the form of calcium dihydroxide precipitated with carbon dioxide during sugar juice purification (British Sugar PLC, Peterborough, UK).

[4]Fiber Sales and Development Corp., Urbana, OH.

[5]The vitamin-micromineral premix provided the following quantities of vitamins and micro minerals per kilogram of complete diet: vitamin A as retinyl acetate, 11,136 IU; vitamin $D_3$ as cholecalciferol, 2,208 IU; vitamin E as DL-alpha tocopheryl acetate, 66 IU; vitamin K as menadione dimethylprimidinol bisulfite, 1.42 mg; thiamin as thiamine mononitrate, 0.24 mg; riboflavin, 6.59 mg; pyridoxine as pyridoxine hydrochloride, 0.24 mg; vitamin $B_{12}$, 0.03 mg; D-pantothenic acid as D-calcium pantothenate, 23.5 mg; niacin, 44.1 mg; folic acid, 1.59 mg; biotin, 0.44 mg; Cu, 20 mg as copper sulfate; Fe, 126 mg as iron sulfate; I, 1.26 mg as ethylenediamine dihydriodide; Mn, 60.2 mg as manganous sulfate; Se, 0.25 mg as sodium selenite and selenium yeast; and Zn, 124.9 mg as zinc sulfate.

**Table 3.** Chemical composition of experimental diets without microbial phytase, as-fed basis

| | Diet[1] | | | | | | |
|---|---|---|---|---|---|---|---|
| Item | Basal | MCP | DCP | Calcium carbonate | *L. calcareum* Ca | Sugar beet co-product | Ca-free |
| DM, % | 90.23 | 90.24 | 90.04 | 90.22 | 89.90 | 89.32 | 92.79 |
| Ash, % | 3.43 | 4.58 | 5.02 | 5.28 | 4.82 | 5.09 | 2.34 |
| GE, kcal/kg | 4,107 | 4,101 | 4,040 | 4,089 | 4,080 | 4,022 | 4,064 |
| CP, % | 13.27 | 13.12 | 12.22 | 12.62 | 12.76 | 12.90 | 8.41 |
| AEE,[2] % | 5.75 | 5.71 | 5.70 | 5.87 | 5.12 | 4.69 | 5.78 |
| ADF, % | 2.64 | 2.55 | 2.56 | 2.51 | 2.64 | 2.41 | 4.56 |
| NDF, % | 9.99 | 9.34 | 8.91 | 8.76 | 9.28 | 9.15 | 8.61 |
| Ca, % | 0.33 | 0.70 | 0.79 | 0.75 | 0.85 | 0.86 | 0.02 |
| P, % | 0.42 | 0.64 | 0.67 | 0.40 | 0.42 | 0.42 | 0.23 |
| Phytase, FTU/kg[3] | <50 | <50 | <50 | <50 | <50 | <50 | <50 |
| Phytate,[4] % | 0.60 | 0.60 | 0.60 | 0.60 | 0.60 | 0.60 | 0.03 |
| Phytate-bound P,[5] % | 0.17 | 0.17 | 0.17 | 0.17 | 0.17 | 0.17 | 0.01 |
| Non-phytate P,[6] % | 0.25 | 0.47 | 0.50 | 0.23 | 0.25 | 0.25 | 0.22 |

[1]MCP = monocalcium phosphate; DCP = dicalcium phosphate. *L. calcareum* Ca is the source of Ca produced by *Lithothamnium calcareum* (Celtic Sea Minerals, Currabinny, Co. Cork, Ireland). The sugar beet co-product is a source of Ca in a form of calcium dihydroxide precipitated with carbon dioxide during sugar juice purification (British Sugar PLC, Peterborough, UK).

[2]AEE = acid-hydrolyzed ether extract.

[3]FTU = phytase units.

[4]Phytate values were calculated from analyzed phytate in the ingredients.

[5]Phytate-bound P was calculated as 28.2% of phytate (Tran and Sauvant, 2004).

[6]Non-phytate P was calculated as the difference between total P and phytate-bound P.

MSP, all diets, and all fecal samples were analyzed for DM (Method 930.15; AOAC, 2007) and for Ca and P by inductively coupled plasma spectroscopy-optical emission spectoscopy (Method 985.01 A, B, and D; AOAC, 2007) after wet ash sample preparation (Method 975.03 B(b); AOAC, 2007). All ingredients and diets were also analyzed for ash (Method 942.05; AOAC, 2007). Corn, potato protein isolate, and diets were analyzed for GE using an isoperibolic bomb calorimeter (Model 6300, Parr Instruments, Moline, IL) and for CP using the combustion procedure (Method 990.03; AOAC, 2007) on an Elementar Rapid N-cube protein/nitrogen apparatus (Elementar Americas Inc., Mt. Laurel, NJ). These samples were also analyzed for ADF (Method 973.18; AOAC, 2007), NDF (Holst, 1973), phytic acid (Ellis et al., 1977), and for acid-hydrolyzed ether extract using 3*N* HCl (Sanderson, 1986) followed by crude fat extraction with petroleum ether (Method 2003.06, AOAC, 2007) on a Soxtec 2050 automated analyzer (FOSS North America, Eden Prairie, MN). Diets were also analyzed for phytase activity (Engelen et al., 2001).

### *Calculations and Statistical Analysis*

The amount of phytate-bound P in corn, potato protein isolate, and diets was calculated as 28.2% of the concentration of phytate (Tran and Sauvant, 2004) and the amount of non-phytate P was calculated as the difference between phytate-bound P and total P. Values for ATTD and STTD of Ca were calculated for each diet according to standard procedures (NRC, 2012). Samples from pigs fed the Ca-free diet were used to determine basal endogenous losses of Ca. The ATTD of Ca in each of the Ca-containing ingredients without or with microbial phytase was calculated using the difference procedure (Adeola, 2001). The values for ATTD of Ca were corrected for endogenous losses of Ca to obtain the STTD of Ca using the same principles as those outlined to calculated standardized ileal digestibility of AA (Stein et al., 2007).

Normality of residuals and outliers were tested using the UNIVARIATE procedure of SAS (SAS Inst. Inc., Cary, NC). Data for the diets were analyzed as a 6 × 2 factorial and the ATTD and STTD of Ca in the Ca sources were analyzed as a 5× 2 factorial using the MIXED procedure of SAS. The model included the fixed effect of diet or Ca source, the level of phytase, the interaction between diet or Ca source and level of phytase, and the random effect of block. Least squares means were calculated for each treatment using the LSMeans procedure in SAS and differences among means were separated using the PDIFF option of SAS. The pig was the experimental unit and an alpha level of 0.05 was used to determine significance among means.

## RESULTS

### *Nutrient Composition of Ingredients and Diets*

The concentration of Ca in corn, potato protein isolate, MCP, DCP, Ca carbonate, *L. calcareum* Ca, and

**Table 4.** Chemical composition of experimental diets with microbial phytase, as-fed basis

| | Diet with 500 FTU/kg[1] | | | | | |
|---|---|---|---|---|---|---|
| Item | Basal | MCP | DCP | Calcium carbonate | *L. calcareum* Ca | Sugar beet co-product |
| DM, % | 89.67 | 90.23 | 90.22 | 90.33 | 90.14 | 89.89 |
| Ash, % | 3.55 | 3.10 | 5.40 | 3.91 | 4.93 | 4.39 |
| GE, kcal/kg | 4,145 | 4,071 | 4,082 | 4,087 | 4,020 | 4,064 |
| CP, % | 12.30 | 12.93 | 13.01 | 13.38 | 12.49 | 12.74 |
| AEE,[2] % | 5.89 | 5.58 | 5.76 | 5.62 | 5.05 | 4.69 |
| ADF, % | 2.46 | 2.36 | 2.19 | 2.36 | 2.47 | 2.42 |
| NDF, % | 8.61 | 9.19 | 9.08 | 9.06 | 9.20 | 9.45 |
| Ca, % | 0.31 | 0.75 | 0.86 | 0.82 | 0.83 | 0.83 |
| P, % | 0.40 | 0.69 | 0.67 | 0.44 | 0.43 | 0.42 |
| Phytase, FTU/kg[3] | 470 | 443 | 431 | 679 | 475 | 544 |
| Phytate,[4] % | 0.60 | 0.60 | 0.60 | 0.60 | 0.60 | 0.60 |
| Phytate-bound P,[5] % | 0.17 | 0.17 | 0.17 | 0.17 | 0.17 | 0.17 |
| Non-phytate P,[6] % | 0.23 | 0.52 | 0.50 | 0.27 | 0.26 | 0.25 |

[1]MCP = monocalcium phosphate; DCP = dicalcium phosphate. *L. calcareum* Ca is a source of Ca produced by *Lithothamnium calcareum* (Celtic Sea Minerals, Currabinny, Co. Cork, Ireland). The sugar beet co-product is a source of Ca in the form of calcium dihydroxide precipitated with carbon dioxide during sugar juice purification (British Sugar PLC, Peterborough, UK).

[2]AEE = acid-hydrolyzed ether extract.

[3]FTU = phytase units.

[4]Phytate values were calculated from analyzed phytate in the ingredients.

[5]Phytate-bound P was calculated as 28.2% of phytate (Tran and Sauvant, 2004).

[6]Non-phytate P was calculated as the difference between total P and phytate-bound P.

sugar beet co-product was between 0.01 and 41.82%, and the concentration of P was between 0.15 and 21.60% (as-fed basis; Table 1). The MSP that was used in this experiment analyzed 0.09% Ca and 27.10% P. The ingredient composition of the experimental diets without microbial phytase is presented in Table 2 (as-fed basis). The concentration of Ca in basal diets without and with microbial phytase was 0.33 and 0.31%, respectively, and the concentration of P was 0.42 and 0.40%, respectively (Tables 3 and 4). The analyzed concentration of Ca in diets containing MCP, DCP, Ca carbonate, *L. calcareum* Ca, and the sugar beet co-product was between 0.70 and 0.86% if phytase was not used and between 0.75 and 0.86% in diets that contained microbial phytase. The concentration of P was between 0.40 and 0.67% if phytase was not used and between 0.42 and 0.69% if phytase was used. The concentration of Ca and P in the Ca-free diet was 0.02 and 0.23%, respectively. The concentration of Ca and P in the diets was close to expected values, with the exception that the concentration of Ca in the Ca carbonate diet without phytase was slightly less than expected, but it was assumed that this small difference did not affect the results. The concentration of phytase in diets was close to expected values.

### *Digestibility of Ca in Diets*

All pigs remained healthy and consumed their assigned diets without apparent problems. The ADFI and basal endogenous losses (mg/d) of Ca were not affected by Ca source or microbial phytase (Table 5). The intake of Ca increased ($P < 0.001$) in pigs fed diets containing DCP or Ca carbonate if microbial phytase was used, but Ca intake was not affected by microbial phytase in the basal diet or in the diets containing MCP, *L. calcareum* Ca, or the sugar beet co-product (Ca source × phytase interaction, $P < 0.001$). Regardless of phytase inclusion, pigs fed diets containing DCP or the sugar beet co-product had greater ($P < 0.05$) fecal output than pigs fed Ca carbonate or the basal diets, but fecal output was not different from MCP and *L. calcareum* Ca. Regardless of Ca source, pigs fed diets containing microbial phytase had greater ($P < 0.05$) fecal output than pigs fed diets that did not contain microbial phytase. Regardless of phytase inclusion, the concentration of Ca was greater ($P < 0.05$) in feces from pigs fed Ca carbonate, *L. calcareum* Ca, or sugar beet co-product than from pigs fed the basal, MCP, or DCP diets. Inclusion of microbial phytase decreased ($P < 0.05$) the concentration of Ca in feces, regardless of the Ca source. The amount of Ca output was not affected by the inclusion of microbial phytase, but pigs fed the *L. calcareum* Ca or the sugar beet co-product diets had a greater ($P < 0.05$) amount of Ca output than pigs fed the basal, MCP, DCP, or Ca carbonate diets. The amount of Ca absorbed in pigs fed the basal, *L. calcareum* Ca, or the sugar beet co-product diets was not affected by microbial phytase inclusion, but pigs fed MCP, DCP, or Ca carbonate had a greater ($P < 0.05$) amount of Ca absorbed if microbial phytase was included in the diets than if no microbial phytase was used (Ca

**Table 5.** Apparent and standardized total tract digestibility (ATTD and STTD) of Ca in diets containing calcium supplements without or with microbial phytase[1]

| Item | ADFI, g/d | Ca intake, g/d | Fecal output, Ca in feces, g/d | % | Ca output, g/d | Ca absorbed, g/d | ATTD of Ca, % | Basal endogenous losses of Ca, mg/d | STTD[2] of Ca, % |
|---|---|---|---|---|---|---|---|---|---|
| No phytase | | | | | | | | | |
| Basal[3] | 757 | 2.54$^{e}$ | 73.27 | 1.20 | 0.91 | 1.63$^{e}$ | 65.51 | 84.07 | 68.88 |
| MCP[4] | 790 | 5.53$^{cd}$ | 81.73 | 1.71 | 1.40 | 4.13$^{c}$ | 75.09 | 87.72 | 76.68 |
| DCP[5] | 758 | 5.97$^{bc}$ | 85.80 | 2.05 | 1.74 | 4.23$^{bc}$ | 71.28 | 83.94 | 72.68 |
| Calcium carbonate | 687 | 5.14$^{d}$ | 69.43 | 2.79 | 1.93 | 3.21$^{d}$ | 62.56 | 76.22 | 64.04 |
| *L. calcareum* Ca[6] | 749 | 6.35$^{ab}$ | 80.65 | 2.84 | 2.28 | 4.07$^{c}$ | 64.06 | 82.90 | 65.36 |
| Sugar beet co-product[7] | 743 | 6.37$^{ab}$ | 87.19 | 2.56 | 2.17 | 4.20$^{bc}$ | 66.12 | 81.69 | 67.40 |
| With phytase | | | | | | | | | |
| Basal[3] | 770 | 2.43$^{e}$ | 76.57 | 0.74 | 0.59 | 1.83$^{e}$ | 76.46 | 84.93 | 80.02 |
| MCP[4] | 792 | 5.93$^{bc}$ | 85.14 | 1.52 | 1.28 | 4.65$^{ab}$ | 78.56 | 87.96 | 80.04 |
| DCP[5] | 783 | 6.72$^{a}$ | 90.08 | 2.02 | 1.81 | 4.90$^{a}$ | 73.51 | 86.99 | 74.80 |
| Calcium carbonate | 763 | 6.24$^{ab}$ | 85.33 | 2.26 | 1.89 | 4.35$^{bc}$ | 69.87 | 84.84 | 71.23 |
| *L. calcareum* Ca[6] | 760 | 6.29$^{ab}$ | 84.27 | 2.48 | 2.07 | 4.22$^{bc}$ | 67.22 | 84.28 | 68.56 |
| Sugar beet co-product[7] | 748 | 6.20$^{b}$ | 88.53 | 2.46 | 2.20 | 4.00$^{c}$ | 65.09 | 82.79 | 66.42 |
| SEM | 41 | 0.30 | 6.22 | 0.17 | 0.17 | 0.23 | 2.33 | 4.58 | 2.33 |
| *P*-value | | | | | | | | | |
| Ca source | 0.086 | 0.003 | 0.007 | <0.001 | <0.001 | <0.001 | <0.001 | 0.074 | <0.001 |
| Phytase | 0.092 | <0.001 | 0.026 | <0.001 | 0.160 | <0.001 | <0.001 | 0.082 | <0.001 |
| Ca source × phytase | 0.572 | 0.001 | 0.509 | 0.399 | 0.564 | 0.008 | 0.113 | 0.565 | 0.106 |

$^{a–e}$Within a column, means without a common superscript differ ($P < 0.05$).

[1]Data are means of 8 observations per treatment, except for the basal diet with phytase that had only 7 observations.

[2]Values for standardized total tract digestibility were calculated by correcting apparent total tract digestibility values for basal endogenous losses. Basal endogenous losses were determined from pigs fed the Ca-free diet as 0.123 g/kg of DMI.

[3]Basal diet contained corn, cornstarch, potato protein isolate, soybean oil, calcium carbonate, monosodium phosphate, vitamins, and minerals was formulated to contain 0.33% Ca.

[4]MCP = monocalcium phosphate.

[5]DCP = dicalcium phosphate.

[6]*L. calcareum* Ca is a source of Ca produced by *Lithothamnium calcareum* (Celtic Sea Minerals, Currabinny, Co. Cork, Ireland).

[7]The sugar beet co-product is a source of Ca in the form of calcium dihydroxide precipitated with carbon dioxide during sugar juice purification (British Sugar PLC, Peterborough, UK).

source × phytase interaction, $P < 0.01$). Regardless of phytase inclusion, pigs fed the MCP diets had the greatest ($P < 0.05$) ATTD of Ca, but pigs fed the basal or DCP diets had a greater ($P < 0.05$) ATTD of Ca than pigs fed the Ca carbonate, *L. calcareum* Ca, or the sugar beet co-product diets. Regardless of phytase inclusion, pigs fed MCP diets had greater ($P < 0.05$) STTD of Ca than pigs fed the DCP, Ca carbonate, *L. calcareum* Ca, or the sugar beet co-product diets, but STTD of Ca was not different between the MCP and basal diets. Regardless of Ca source, the ATTD and STTD of Ca increased ($P < 0.05$) if microbial phytase was added to the diets.

### *Digestibility of Ca in Ingredients*

Inclusion of microbial phytase did not affect the ATTD or STTD of Ca in the Ca sources (Table 6). Regardless of inclusion of microbial phytase, pigs fed MCP had the greatest ($P < 0.05$) ATTD and STTD values of Ca, and pigs fed DCP had greater ($P < 0.05$) ATTD and STTD of Ca than pigs fed Ca carbonate, *L. calcareum* Ca, or the sugar beet co-product, but no differences were observed among ATTD and STTD of Ca in Ca carbonate, *L. calcareum* Ca, and the sugar beet co-product.

### *Digestibility of P in Diets*

Regardless of Ca source, inclusion of microbial phytase increased ($P < 0.05$) the amount of P intake, percentage of P absorbed, and ATTD of P (Table 7). The percentage of P in the feces and amount of P output decreased ($P < 0.05$) if microbial phytase was included in the diets. Regardless of phytase inclusion, pigs fed MCP or DCP had greater ($P < 0.05$) P intake than pigs fed the basal, Ca carbonate, *L. calcareum* Ca, or the sugar beet co-product diets. Pigs fed the basal diets had less ($P < 0.05$) concentration of P in feces than pigs fed MCP, DCP, Ca carbonate, *L. calcareum* Ca, or the sugar beet co-product diets, regardless of phytase inclusion. The amounts of P output from pigs fed MCP, DCP, *L. calcareum* Ca, or the sugar beet co-product were greater ($P <$ 0.05) than from pigs fed Ca carbonate or the basal diets,

regardless of phytase inclusion, but pigs fed Ca carbonate diets had greater ($P < 0.05$) P output than pigs fed the basal diets. The percentage of P absorbed was greater ($P < 0.05$) in pigs fed MCP or DCP diets than pigs fed basal, Ca carbonate, *L. calcareum* Ca, or the sugar beet co-product diets, but pigs fed basal diets had greater ($P < 0.05$) percentage of P absorbed than pigs fed Ca carbonate, *L. calcareum* Ca, or the sugar beet co-product diets. The ATTD of P was greater ($P < 0.05$) in pigs fed basal, MCP, or DCP diets than pigs fed Ca carbonate, *L. calcareum* Ca, or the sugar beet co-product diets, regardless of phytase inclusion, but pigs fed Ca carbonate had greater ($P < 0.05$) ATTD of P than pigs fed *L. calcareum* Ca or the sugar beet co-product diets.

## DISCUSSION

The concentration of Ca and P in MCP and the concentration of P in DCP used in this experiment are in agreement with reported values (Sauvant et al., 2004; Rostagno et al., 2011; NRC, 2012), but the concentration of Ca in DCP was less than published values (Sauvant et al., 2004; Rostagno et al., 2011; NRC, 2012). The reason for variations in the concentration of Ca in MCP and DCP is that what are commercially known as MCP and DCP in fact are mixtures of a number of P-containing compounds (Baker, 1989; Petersen and Stein, 2006) as a result of the way feed-grade MCP and DCP are produced. Production of MCP and DCP starts with the addition of phosphoric acid to Ca carbonate and the reaction between these 2 components is stopped according to the amount of total P desired in the final product. The reaction will be stopped at 18.5% P if DCP is produced and at 21.0% P if MCP is produced. Therefore, the final product is a mixture of unreacted calcium carbonate, MCP, hydrated DCP, anhydrated DCP, and other reactive P compounds. Phosphorus compounds such as ferrous phosphate, aluminum phosphate, magnesium phosphate, sodium phosphate, and unreacted phosphoric acid represent between 15 and 17% of the total P in the final products (Baker, 1989). Calcium is also present as Ca fluoride and Ca sulfate, which represents 36.2 to 42.3% of the total Ca in the final products (Baker, 1989). In general, what is commercially known as MCP contains 50 to 70% MCP and 10 to 15% DCP, and DCP may contain approximately 29% MCP and 57% DCP (Petersen and Stein, 2006).

The concentrations of Ca and P in Ca carbonate used in this experiment were greater than reported values (Sauvant et al., 2004; NRC 2012), but the concentrations of Ca and P in MSP are within the range of reported values (Sauvant et al., 2004; González-Vega et al., 2013, 2014; NRC, 2012). *Lithothamnium calcareum* may be used as a Ca supplement for poultry (Walk et al., 2012) and pigs (Melo and Moura, 2009; González-Vega et al., 2014). The concentrations of Ca and P in *L. calcareum* Ca used in this experiment were slightly greater than reported values (Melo and Moura, 2009; Walk et al., 2012; González-Vega et al., 2014).

The sugar beet co-product is mostly used in agriculture for soil conditioning and for correction of soil acidity (Associated British Foods Annual Report and Accounts, 2008). The concentrations of Ca and P in the sugar beet co-product used in this experiment were close to expected values.

The concentration of Ca and P in corn concur with reported values (Sauvant et al., 2004; Rostagno et al., 2011; Almeida and Stein, 2012; NRC, 2012), but the concentration of phytate-bound P was slightly less than reported values (Almeida and Stein, 2012; Sauvant et al., 2004). The concentration of Ca, P, and phytate in potato protein isolate were close to previous data (González-Vega et al., 2013). The concentrations of Ca and P in the diets were close to expected values, but varied among diets due to the way the diets were

**Table 6.** Apparent and standardized total tract digestibility (ATTD and STTD)of Ca in calcium supplements without or with microbial phytase[1]

| Item | ATTD of Ca, % | STTD[2] of Ca, % |
|---|---|---|
| No phytase | | |
| MCP[3] | 82.76 | 85.86 |
| DCP[4] | 75.29 | 77.80 |
| Calcium carbonate | 57.98 | 60.43 |
| *L. calcareum* Ca[5] | 62.54 | 64.98 |
| Sugar beet co-product[6] | 66.18 | 68.41 |
| With phytase | | |
| MCP[3] | 83.24 | 86.34 |
| DCP[4] | 76.39 | 78.90 |
| Calcium carbonate | 70.62 | 73.07 |
| *L. calcareum* Ca[5] | 66.24 | 68.67 |
| Sugar beet co-product[6] | 63.18 | 65.41 |
| SEM | 3.80 | 3.80 |
| *P*-value | | |
| Ca source | <0.001 | <0.001 |
| Phytase | 0.173 | 0.173 |
| Ca source × phytase | 0.212 | 0.212 |

[1]Data are means of 8 observations per treatment.

[2]Values for standardized total tract digestibility were calculated by correcting apparent total tract digestibility values for basal endogenous losses. Basal endogenous losses were determined from pigs fed the Ca-free diet as 0.123 g/kg of DMI.

[3]MCP = monocalcium phosphate.

[4]DCP = dicalcium phosphate.

[5]*L. calcareum* Ca is a source of Ca produced by *Lithothamnium calcareum* (Celtic Sea Minerals, Currabinny, Co. Cork, Ireland).

[6]The sugar beet co-product is a source of Ca in the form of calcium dihydroxide precipitated with carbon dioxide during sugar juice purification (British Sugar PLC, Peterborough, UK).

**Table 7.** Apparent total tract digestibility (ATTD) of P in diets containing different calcium supplements without or with microbial phytase[1]

| Item | P intake, g/d | P in feces, % | P output, g/d | P absorbed, % | ATTD of P, % |
|---|---|---|---|---|---|
| No phytase | | | | | |
| Basal[2] | 3.19 | 1.47 | 1.09 | 2.09 | 66.14 |
| MCP[3] | 5.04 | 1.93 | 1.56 | 3.48 | 69.03 |
| DCP[4] | 5.05 | 1.78 | 1.48 | 3.57 | 70.62 |
| Calcium carbonate | 2.76 | 1.76 | 1.23 | 1.53 | 55.27 |
| *L. calcareum* Ca[5] | 3.16 | 1.94 | 1.56 | 1.60 | 50.56 |
| Sugar beet co-product[6] | 3.13 | 1.80 | 1.51 | 1.62 | 51.47 |
| With phytase | | | | | |
| Basal[2] | 3.09 | 1.14 | 0.87 | 2.22 | 72.17 |
| MCP[3] | 5.44 | 1.57 | 1.32 | 4.12 | 75.75 |
| DCP[4] | 5.23 | 1.66 | 1.50 | 3.73 | 71.68 |
| Calcium carbonate | 3.36 | 1.51 | 1.27 | 2.10 | 62.29 |
| *L. calcareum* Ca[5] | 3.27 | 1.59 | 1.33 | 1.95 | 59.45 |
| Sugar beet co-product[6] | 3.17 | 1.60 | 1.35 | 1.81 | 57.28 |
| SEM | 0.22 | 0.11 | 0.11 | 0.15 | 2.01 |
| *P*-value | | | | | |
| Ca source | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| Phytase | 0.004 | <0.001 | 0.002 | <0.001 | <0.001 |
| Ca source × phytase | 0.065 | 0.566 | 0.142 | 0.103 | 0.358 |

[1]Data are means of 8 observations per treatment, except for the basal diet with phytase and the sugar beet co-product diet with phytase, which had only 7 observations.

[2]Basal diet contained corn, cornstarch, potato protein isolate, soybean oil, calcium carbonate, monosodium phosphate, vitamins, and minerals was formulated to contain 0.33% Ca and 0.26% standardized total tract digestible P.

[3]MCP = monocalcium phosphate.

[4]DCP = dicalcium phosphate.

[5]*L. calcareum* Ca is a source of Ca produced by *Lithothamnium calcareum* (Celtic Sea Minerals, Currabinny, Co. Cork, Ireland).

[6]The sugar beet co-product is a source of Ca in the form of calcium dihydroxide precipitated with carbon dioxide during sugar juice purification (British Sugar PLC, Peterborough, UK).

formulated. Because of the different concentration of Ca and P in the test ingredients used in this experiment, it was not possible to maintain a constant concentration of Ca and P among all diets. However, the ATTD of Ca is not influenced by Ca concentration in the diets if the Ca concentration is between 0.33 and 0.80% (Stein et al., 2011) and if the P concentration is between 0.44 and 0.66% (Stein et al., 2008), and all diets, except the Ca-free diet, were formulated to stay within these limits.

### *Phytate and Ca Interactions*

Phytate in plant feed ingredients may bind up to 6 atoms of Ca and promote the formation of Ca-phytate complexes and reduce the digestibility of Ca in the intestinal tract (Selle et al., 2009). There are 2 possible mechanisms that may explain how phytate decreases the digestibility of Ca. One possible mechanism is that phytate binds the intrinsic Ca in the feed ingredient and reduces digestibility. For example, when canola meal was used as the sole source of dietary Ca, the ATTD and true total tract digestibility of Ca were less than that of pigs fed canola meal with microbial phytase (González-Vega et al., 2013). This indicates that intrinsic Ca in canola meal was indeed bound to phytate, and the addition of microbial phytase hydrolyzed the phytate ester bonds and, therefore, phytate had less ability to bind Ca if phytase was added to the diet. The other possible mechanism is that not only intrinsic Ca but also supplemented dietary Ca may form complexes with phytate. The ATTD of Ca in diets in which the majority of the Ca was inorganic Ca increased if phytase was added to the diets (Guggenbuhl et al., 2007; Poulsen et al., 2010; Rodríguez et al., 2013). The increase in ATTD of Ca in most of these experiments was greater than what can be explained by release of intrinsic Ca from phytate, which indicates that some of the added dietary Ca may also complex with phytate if phytase is not used in the diet. The fact that the ATTD and STTD of Ca in the diets evaluated in this experiment increased as microbial phytase was added to the diet indicates that some of the Ca in the feed ingredients was bound to phytate. The observation that microbial phytase did not affect the ATTD and STTD of Ca in the Ca supplements, but only in the diets, further indicates that dietary phytate binds to Ca from the inorganic dietary Ca sources.

### *Differences in Digestibility of Ca among Sources of Ca*

The greater ATTD and STTD of Ca in MCP and DCP than in Ca carbonate indicates that Ca in Ca carbonate is more easily bound to phytate, less soluble, or is a less digestible Ca source than Ca from MCP and DCP. This is likely because Ca in MCP and DCP is already bound to P, which is a result of the reaction between Ca carbonate and phosphoric acid in the production of these ingredients.

We are not aware of other experiments with pigs in which Ca digestibility is compared among MCP, DCP, and calcium carbonate, but in a recent experiment with broilers it was observed that the digestibility of Ca in MCP (67.9%) is greater than in limestone (34.1%; Angel, 2013). Thus, results obtained both with broilers and with pigs indicate that the digestibility of Ca in Ca carbonate or limestone is less than the digestibility of Ca in MCP and DCP.

Concentrations of Ca in *L. calcareum* Ca and the sugar beet co-product were relatively less than in Ca carbonate; however, *L. calcareum* Ca is a very soluble source of Ca (Walk et al., 2012; González-Vega et al., 2014). The ATTD of Ca in Ca carbonate obtained in this experiment was in agreement with reported values (Stein et al., 2011), but the ATTD and STTD of Ca in Ca carbonate and *L. calcareum* Ca were greater than the values reported by González-Vega et al. (2014). The total and available P levels in the diets were similar among experiments, but the main difference among the experiments is that corn-based diets were used by Stein et al. (2011) and in the present experiment, whereas semi-synthetic diets were used by González-Vega et al. (2014). We are not aware of the form in which Ca is present in *L. calcareum* Ca or in the sugar beet co-product, but the observation that ATTD and STTD of Ca in *L. calcareum* Ca and the sugar beet co-product were not different from values for Ca carbonate without or with microbial phytase indicates that Ca-P complexes may have been formed in pigs fed *L. calcareum* Ca (González-Vega et al., 2014) and the sugar beet co-product.

A difference in relative bioavailability among Ca supplements has been observed (Ross et al., 1984) and results of this experiment demonstrated that there are differences in STTD of Ca among Ca supplements. As a consequence, use of the generated values for STTD of Ca in Ca supplements without or with microbial phytase may result in more accurate formulation of diets fed to pigs.

### *Digestibility of P*

Phosphorus from corn and potato protein isolate represents 30 to 53% of the total P in the diets used in this experiment. Because some of the P in corn and potato protein isolate is bound to phytate, 24 to 43% of the total P in the diets was bound to phytate. As a result of hydrolysis of phytate by phytase, inclusion of microbial phytase in the diets decreases the amount of P excreted in feces (Harper et al., 1997; Poulsen et al., 2010). Therefore, the increase in ATTD of P that was observed as phytase was added to the diets is a result of release of P bound to the phytate in corn and potato protein isolate.

Monosodium phosphate was the source of P used in the basal, Ca carbonate, *L. calcareum* Ca, and sugar beet co-product diets and MCP and DCP were the main sources of P in the MCP and DCP diets. The ATTD of P in MSP is greater than in MCP and DCP (Petersen and Stein, 2006), but in the current experiment, the greater ATTD of P observed in diets containing MCP or DCP compared with diets using MSP may be a result of the reduced contribution of P from corn in diets containing MCP or DCP compared with diets containing MSP.

### *Conclusions*

The ATTD and STTD of Ca were greater in MCP and DCP than in Ca carbonate, *L. calcareum* Ca, and the sugar beet co-product. Inclusion of microbial phytase increased the ATTD and STTD of Ca in diets, but not in the Ca supplements, confirming that dietary phytate interferes with Ca digestibility. The ATTD of P was greater in the basal, MCP, and DCP diets than in the other diets, and regardless of the Ca source, addition of microbial phytase increased the ATTD of P in all diets. Results indicate that diets may be more accurately formulated if values for STTD of Ca rather than values for total Ca are used in diet formulations.

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