Supplemental Dietary Inulin Affects the Bioavailability of Iron in Corn and Soybean Meal to Young Pigs

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Abstract
Iron deficiency represents one of the most common global nutritional disorders in humans. Our objective was to determine whether and how supplemental inulin improved utilization of iron intrinsically present in a corn and soybean meal diet by young pigs for hemoglobin repletion. In Expt. 1, 3 groups (n = 8/group) of pigs were fed a corn and soybean meal–based diet (BD, without inorganic iron addition) or BD + 2 or 4% inulin (Synergy 1: a mixture of oligofructose and long-chain inulin HP, Orafti) for 5 wk. Final blood hemoglobin concentrations and the overall hemoglobin repletion efficiency of pigs were positively (r = 0.55 and 0.69, P < 0.01) correlated with dietary inulin concentrations. Compared with pigs fed the BD, those fed 4% inulin demonstrated a 28% improvement (P < 0.01) in hemoglobin repletion efficiency and 15% (P < 0.01) improvement in the final blood hemoglobin concentration. In Expt. 2, 12 weanling pigs (n = 6/group) were fed the BD or the BD + 4% inulin for 6 wk. Pigs fed 4% inulin had higher (P < 0.05) soluble Fe concentrations in the digesta of the proximal, mid, and distal colon, and lower (P < 0.05) sulfide concentrations in the digesta of the distal colon. Supplemental inulin had virtually no effect on pH or phytase activity of digesta from any of the tested segments. In conclusion, supplementing 4% inulin improved utilization of intrinsic iron in the corn and soybean meal diet by young pigs, and this benefit was associated with soluble Fe and sulfide concentrations but not pH or phytase activity in the digesta. J. Nutr. 136: 3033–3038, 2006.

Introduction
Iron deficiency is the most common nutritional disorder in humans, affecting >2 billion people around the world (1). Although food fortification and iron supplements have been used to effectively combat this problem in certain regions (2), these interventions are difficult to sustain or to reach the most at-risk groups of people. As staple food crops (e.g., rice, wheat, maize, beans, cassava, and sweet potato) are the major dietary sources of iron for people in developing nations, enhancing bioavailable iron in those crops could be the most effective and sustainable strategy to reduce or prevent iron deficiency in these populations (3).

Iron bioavailability of staple crops may be improved by removing or reducing inhibitors of iron utilization (e.g., phytate and polyphenolics) and/or enriching enhancers of iron utilization (4). Recently, inulin and short-chain fructooligosaccharides (FOS) have been studied as possible candidates of such enhancers (5–9). These compounds are unique D-fructofuranose polymers linked by a β2→1 bond at the anomeric C2, and are accumulated in the tissues of many plant species (10). The general assumption is that these compounds are indigestible in the upper digestive tract of simple-stomached animals and humans (11), but that they pass to their lower gut to be fermented by microbes (10).

Positive effects of supplemental inulin or FOS on bioavailability of dietary calcium and magnesium in animals and humans have been reported (12–17). However, only a few studies (5–9,18) have been conducted to determine such effects on bioavailability of dietary iron. Consequently, several major issues remain to be clarified. First, the effects of inulin or FOS on dietary iron utilization are inconclusive, as benefits to blood hemoglobin (Hb) concentration and hematocrit were shown in rats fed diets containing 5 or 7.5% FOS (6,7), whereas no improvement in iron utilization was produced by supplementation of inulin or FOS in healthy men (8,9). Second, all experimental animals or tested subjects in past studies were fed inorganic iron supplements (6,7,18). Thus, the exclusive effects of supplemental inulin or FOS on the bioavailability of iron intrinsically present in foods of plant origin have not been studied. Lastly, there is little information on the mode of action of inulin for its possible enhancement of iron bioavailability.

Because in vivo and in vitro studies have demonstrated that inulin and FOS stimulated the proliferation of certain types of colonic bacteria, such as bifidobacteria and lactobacilli (19–26),
most inulin studies on mineral nutrition have been focused on the possible changes of these microbial populations (31,32) except for iron (no inorganic iron was added). The actual concentrations of iron in all experimental diets were determined using the method described by Quemer et al. (34) (Table 1). Synergy 1 (Orafti) was used as the source of inulin replacing corn starch in the basal diet (BD). This product was a mixture of α-D-glucopyranosyl-β-D-fructofuranosyl)n-1-β-D-fructofuranosides (n = 10–60, mean of 25), and oligofructose, α-D-fructopyranosyl-(β-D-fructofuranosyl)n-1-β-D-fructofuranosides (n = 2–7, mean of 4).

Experimental animals and protocols. Two experiments were conducted with a total of 36 weaning Yorkshire × Hampshire × Landrace crossbred pigs from the Cornell University Swine Farm. Both experiments were approved by the University Institutional Animal Care and Use Committee. All experimental pigs were selected from litters that contained adequate concentrations of all nutrients (32) except for iron (no inorganic iron was added). The actual concentrations of iron in all experimental diets were analyzed using an inductively coupled argon plasma emission spectrometer (ICAP 61E Trace Analyzer, Thermo Electron) (33). The actual concentrations of iron in all experimental diets were determined using the method described by Quemer et al. (34) (Table 1). Synergy 1 (Orafti) was used as the source of inulin replacing corn starch in the basal diet (BD). This product was a mixture of α-D-glucopyranosyl-β-D-fructofuranosyl)n-1-β-D-fructofuranosides (n = 10–60, mean of 25), and oligofructose, α-D-fructopyranosyl-(β-D-fructofuranosyl)n-1-β-D-fructofuranosides (n = 2–7, mean of 4).

Materials and Methods

Basal diet and inulin. The basal diet consisted of corn and soybean meal (Table 1), and contained adequate concentrations of all nutrients except for iron (no inorganic iron was added). The actual concentrations of iron in all experimental diets were analyzed using an inductively coupled argon plasma emission spectrometer (ICAP 61E Trace Analyzer, Thermo Electron) (33). The actual concentrations of iron in all experimental diets were determined using the method described by Quemer et al. (34) (Table 1). Synergy 1 (Orafti) was used as the source of inulin replacing corn starch in the basal diet (BD). This product was a mixture of α-D-glucopyranosyl-β-D-fructofuranosyl)n-1-β-D-fructofuranosides (n = 10–60, mean of 25), and oligofructose, α-D-fructopyranosyl-(β-D-fructofuranosyl)n-1-β-D-fructofuranosides (n = 2–7, mean of 4).

Growth performance and sample collection. In both experiments, feed intake of individual pigs was recorded daily and body weight of individual pigs was measured weekly. Blood samples of all individual pigs (fasted overnight for 8 h) were collected weekly from the anterior vena cava using 5-mL heparin syringes to assay for blood Hb and hematocrit. At the end of Expt. 2, all pigs were killed by electrical stunning and exsanguinations. Based on a preliminary experiment, pigs were fistled for 8 h and then were given free access to feed for 10 h prior to slaughter for us to collect comparable and sufficient digesta samples from all designated segments. The digestive tracts were quickly removed from the carcass and separated into various sections for digesta sampling. Digesta samples for the stomach were collected from the entire contents and thoroughly mixed using a blender. Digesta samples for different parts of intestines were collected from a 12 cm segment each, and the excisions were as follows: upper jejunum, 2 m posterior to the pylorus; lower jejunum, 2 m anterior to the ileo-caecal junction; proximal colon, immediately posterior to the ileo-caecal junction; mid colon, equal length up and down the mid transverse colon; and distal colon, immediately anterior to the rectum. The samples were immediately frozen in liquid nitrogen, and stored in a −20°C freezer. After 48 h, all samples were freeze-dried (20 SRC-X, Virtis) and stored in a −20°C freezer until analysis. All the assayed values were expressed on a dry matter basis, and moisture contents in the fresh digesta samples were calculated from the weight difference before and after freeze drying.

Blood sample analyses. Blood Hb concentrations were measured spectrophotometrically using the cyanomethemoglobin method following the manufacturer’s instructions (Pointe Scientific). Hematocrit values were determined using heparinized microcapillary tubes (Fisher

<table>
<thead>
<tr>
<th>Ingredient</th>
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<th>+ 2% Inulin</th>
<th>+ 4% Inulin</th>
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<tr>
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<td>40.0</td>
</tr>
<tr>
<td>Total</td>
<td>1000</td>
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1. Vitamin and mineral premix provided/kg diet: retinyl palmitate, 1208 μg; ergocalcif- erol, 5.5 μg; d-α-tocopherol acetate, 10.72 mg; menadione, 0.5 mg; d-biotin, 0.05 mg; choline chloride, 0.5 g; folic acid, 0.3 mg; niacin, 15 mg; Ca-D-panthothenate, 10 mg; riboflavin, 3.5 mg; thiamin 1 mg; pyridoxine, 1.5 mg; Cyanocobalamin, 17.5 μg; CuSO4 7H2O, 6 mg; CaH2N2H2, 6 mg; ethylene diamine dihydroiodide, 0.14 mg; MnO, 4 mg; Na2SO4 0.3 mg, ZnO, 100 mg.
2. Calculated based on NRC.
3. Analyzed using an ICAP 61E Trace Analyzer, Thermo Electron.
4. Analyzed using the method described by Quemer et al. (34).
Hemoglobin repletion efficiency (HRE) was determined using the following formula (35):

\[
\text{HRE} = \frac{[\text{final total body Hb Fe, mg} - \text{initial total body Hb Fe, mg}]}{(\text{total Fe intake, mg})} \times 100.
\]

and total body Hb iron content was estimated using the following formula:

\[
\text{Hb Fe, mg} = [\text{body weight (g)} \times 0.067 \text{mL blood/g BW}] \\
\times (\text{Hb, g/mL}) \times (3.35 \text{mg Fe/Hb, g}).
\]

**Digesta sample analyses.** Total digesta Fe concentration was measured using the same method for dietary Fe concentrations. To determine pH and soluble iron of digesta and fecal samples, 2 g of fresh wet samples were suspended in 18 mL of distilled water and mixed on an rotator stirrer for 30 min at the room temperature and centrifuged at 3,000 \( \times g \) for 15 min at 4°C (GS-6KR Centrifuge, Beckman Instruments). The pH in the homogenates was determined using a glass electrode (Accumet Tris Compatible Combination Electrode, Model 630, Fisher Scientific). Soluble iron concentration in the prepared homogenates was measured using a ferrozine assay (36). After 0.1 mL of homogenate was diluted in 0.9 mL of deionized water, 0.1 mL of ferrozine chromogen solution was added for color development. The absorbance was measured at 562 nm using KC-4 version 2.6 microplate scanning spectrophotometer (BIO-TEK Instruments). Total soluble sulfide concentration in the fresh digesta was determined as previously described (37–39). Digesta phytase activities were measured using a spin column method as described by Kim and Lei (40) at 2 pH levels: the actual digesta pH for each segment and the commonly used pH (5.5) for phytase activity assay.

**Statistical analyses.** Data were analyzed as a randomized block design using the Proc General Linear Models procedure of SAS (version 6.12, SAS). Effects of dietary inulin on various measures were analyzed using 1-way ANOVA with or without time-repeated measurements. Dosedependent effects of inulin in Expt. 1 were analyzed using Proc Reg procedure of SAS. Each individually penned pig was used as the experimental unit. The Bonferroni/Dunn t-test was used to compare treatment means, and the significance level was set at \( P \leq 0.05 \) (41). Values in the text are means ± SEM.

**Results**

**Expt. 1.** Whereas the 3 groups of pigs had similar initial blood Hb concentrations at wk 0, final blood Hb concentrations in pigs fed 4% inulin was 15% higher (\( P < 0.05 \)) than pigs fed BD (Table 2). However, the concentration in pigs fed 2% inulin was not statistically different from pigs fed either BD or 4% inulin. The changes in mean Hb concentrations over the 5-wk period between the treatment groups displayed the same statistical outcome as their final blood Hb concentrations (data not shown). Pigs fed 4% inulin had higher (\( P < 0.01 \)) overall HRE than pigs fed BD or 2% inulin (Fig. 1). The improvement noted for pigs fed 2% inulin did not differ from pigs fed the BD. Responses of final blood Hb concentrations and overall HRE to dietary inulin concentrations were described by the following linear regression equations:

\[
\text{Final Hb concentration} = 12.71 + 0.48 \times \% \text{ inulin (} R^2 = 0.30, P < 0.01) \\
\text{Overall HRE} = 22.60 + 2.26 \\
\times \% \text{ inulin (} R^2 = 0.48, P < 0.01). 
\]

Weekly data analysis (not shown) indicated that the inulin effects on HRE became marginally significant at wk 4 (\( P = 0.07 \)) and significant at wk 5 (\( P < 0.01 \)). Dietary inulin concentrations had no effect on final body weight, daily body weight gain, daily feed intake, final hematocrit, or final fecal pH (Table 2).

**Expt. 2.** There was no difference in overall growth performance or final hematocrit between pigs fed BD and 4% inulin. Pigs fed 4% inulin had 14% higher (94.4 ± 4.5 vs. 107.8 ± 3.7 g/L, \( P = 0.06 \)) blood Hb concentrations at wk 6, and 22% higher (20.4 ± 1.4 vs. 24.9 ± 0.7%, \( P < 0.05 \)) overall HRE than pigs fed BD. The changes in Hb concentrations over the 6-wk period were greater (\( P < 0.05 \)) in pigs fed 4% inulin than those fed the BD. Although total iron concentrations of digesta from various segments were not significantly different (Fig. 2A), pigs fed 4% inulin had 45% higher (\( P < 0.01 \)) soluble Fe concentrations in the 3 segments of colon than those of pigs fed BD (Fig. 2B). In contrast, digesta soluble sulfide concentration in pigs fed 4% inulin was 32% lower (\( P < 0.01 \)) in digesta of distal colon and marginally lower (17%, \( P = 0.08 \)) in mid colon than pigs fed BD (Fig. 3). The pH of digesta samples from various segments did not differ between the x and y groups at the end of the study (stomach: 3.2 ± 0.2 vs. 3.3 ± 0.2; upper jejunum: 6.6 ± 0.2 vs. 6.5 ± 0.1; lower jejunum: 7.3 ± 0.1 vs. 7.2 ± 0.1; proximal colon: 6.6 ± 0.2 vs. 6.6 ± 0.2; mid colon: 6.8 ± 0.1 vs. 6.8 ± 0.03; and distal colon: 6.8 ± 0.1 vs. 6.7 ± 0.1) or fecal samples (6.0 ± 0.2 vs. 6.1 ± 0.2). When phytase activity in digesta was assayed at pH 5.5, the 2 groups of pigs did not differ in digesta from any segment except for lower jejunum, where pigs fed 4% inulin affected iron bioavailability.
inulin had a slightly higher activity than pigs fed the BD (Table 3). When phytase activity in digesta was assayed at the actual digesta pH of each segment, only stomach digesta showed detectable activity (BD, 52.9 ± 11.5 units/g; 4% inulin, 29.2 ± 7.5 units/g; \( P = 0.10 \)).

### Discussion

Supplemental inulin added to corn and soybean meal diets significantly improved bioavailability of iron from corn and soybean meal diets fed to weanling pigs. In Expt. 1, this improvement displayed a linear response to dietary inulin dose. Because pigs in both experiments were fed the diets without inorganic iron addition, the improved HRE clearly indicated that supplemental 4% inulin effectively enhanced the bioavailability of iron intrinsically present in corn and soybean meal to meet the most quantitatively important function of iron in the body, hemoglobin synthesis. This finding is novel and extremely encouraging as a strategy for improving human iron nutrition through biofortification of staple food crops (3). Although ingestion of high concentrations of inulin may cause excessive flatus, borborygmi, and bloating (42), earlier studies have shown that humans may be able to tolerate inulin intakes up to 30 g/d (42). Most plants have inulin concentrations ranging from 0.1% to 3.2% (43). If our pig data are applicable to humans, it will be physiologically feasible to achieve significant improvements in iron nutrition of target human populations by enriching inulin in their staple foods via plant breeding. Differential effects of short and long-chain inulin on iron bioavailability should be considered for the target enrichment. The entire plant of such new varieties would need to be thoroughly tested for possible alterations in other nutrients.

The positive effect of inulin on HRE in pigs in the present study is consistent with that of FOS in rats reported by Ohta et al. (6). However, other groups did not observe a positive effect of inulin or FOS in humans (8,9). This discrepancy does not seem to be simply explained by differences in inulin or FOS doses between these experiments. The positive effects in rats was produced by 5–10% inulin or FOS (5–7, 14), whereas human subjects who did not show a response were given ~3% (15 g of inulin/d) (8) or 8% (40 g of inulin/d) (9) inulin. Alternatively, initial iron status of the experimental animals or human subjects might be the key determinant of the treatment outcomes. Our pigs experienced moderate iron-deficient anemia and grew normally, which provided an appropriate physiological condition for inulin to show its effect on iron bioavailability. However, healthy, non-Fe–deficient subjects were used in human studies (8,9).

Thus, supplemental inulin may exert a greater role in iron-deficient animals than in iron-deficient humans (6,8). If so, enriching inulin in staple crops may benefit the iron-deficient population without putting the iron-adequate population at risk of iron excess. As the positive effects of 4% inulin on blood Hb and HRE were not significant until wk 5, a minimal length of time was needed for supplemental inulin to show its maximum effect. In addition, the type of inulin may affect the outcome. The inulin used in our study was a mixture of long and short-chain oligofructose polymers, whereas van den Huvel et al. (8) used short-chain oligofructose consisting of glucose linked to 2–4 fructose units, and Coudray et al. (9) used inulin of longer chain length (DP = 15). Different types of inulin or FOS fare differently in the digestive tracts and affect different types of microbial populations, leading to different digestive or metabolic impacts (22,44–46). It is also interesting to mention that soybean meal contains 4–6% galactooligosaccharides (47). As we and others have still observed an enhanced iron bioavailability by supplementing inulin or FOS into the basal diets containing up to 30% soybean meal, this again suggests the specificity of oligosaccharides in impacting select biochemical and metabolic responses.

Compared with pigs fed the BD, pigs fed 4% inulin had higher concentrations of soluble iron and lower concentrations of sulfide in digesta of the colon. To the best of our knowledge,
the effect of inulin on digesta sulfide concentrations has not been reported, although Sakai et al. (7) observed an increased soluble iron concentration in the colon of rats fed 7.5% short-chain FOS. The increased solubility of iron in colon digesta would promote absorption of iron if mineral absorption takes place in the large intestine (7,16,48). Although it is still a subject of debate as to whether a significant amount of iron can be absorbed in the colon (49), a few recent studies have shown the expression of iron absorption-related genes in the large intestines of rats and mice (50,51). Furthermore, Ohta et al. (48) have reported that dietary inulin supplementation resulted in a positive correlation between apparent calcium absorption and the relative amounts of calbindin (calbindin-D9k) and strongly induced CaBP expression in large intestines of rats. Our group is actively investigating whether inulin can upregulate iron transporter in the colon of pigs.

As sulfide is generated from microbial fermentation (27), the reduced concentration of sulfide in the distal colon digesta may be interpreted as a modified microbial population in the colon of the inulin-fed pigs, leading to an attenuated hydrogen sulfide production (27,52). Consequently, lowering sulfide would reduce its binding to iron (53), leaving more iron soluble or available for possible absorption. Our data are in agreement with Swanson et al. (22) and Flickinger et al. (45) who observed a reduction in fecal hydrogen sulfide and other fecal putrefactive agents (protein fermentative catabolites) in dogs fed FOS. Reduction of such moieties, if verified, would be extremely important in human and companion animal gut health because these subjects may ingest excess protein, and indoles, phenols, and S-containing compounds have large bowel disease implications (27).

Because we did not see an effect of supplemental inulin on fecal or digesta pH, and virtually no effect of supplemental inulin on phytase activity in digesta of various segments at either actual digesta pH or pH 5.5, the inulin-produced improvements in HRE of pigs was not associated with intestinal pH or digesta phytase activity. Reported changes of digesta pH by supplemental inulin or FOS have been controversial. Loh et al. (54) showed an elevated colonic pH in pigs fed 3% inulin, whereas Kleessen et al. (44) observed a decreased cecal and colonic pH in rats fed 5% short or long-chain FOS. Meanwhile, Mikkelsen et al. (55) found no changes of digesta pH in pigs fed 4% FOS. Thus, supplemental inulin or FOS does not always affect digesta pH, and their effect on iron bioavailability is not necessarily associated with lowering intestinal pH. Although stomach digesta had detectable phytase activity at its actual pH, and lower jejunum digesta phytase activity showed an inulin effect at pH 5.5, their activities were low compared with those in colon digesta. Thus, the detected phytase activity in upper gut was due to mainly the plant phytase present in the diet and probably did not have a major impact on iron bioavailability. Clearly, supplemental inulin did not seem to promote phytase-producing microbes in the gastrointestinal tracts of pigs.

In conclusion, our results indicate that supplemental 4% inulin improved utilization of iron intrinsically present in corn and soybean meal by young pigs for hemoglobin synthesis. This positive effect of inulin was associated with deceased concentrations of sulfide and increased concentrations of soluble iron in colon digesta, but not with digesta pH or phytase activity across different segments of the gastrointestinal tracts of pigs. The supplemental inulin concentration used in the present study is close to the tolerable threshold of humans (42), above the level that caused dramatic positive shifts in the composition of microbiota in humans (56), and achievable in staple crops by plant breeding (42). Thus, our findings are highly relevant to improving iron nutrition in anemic population through biofortification. The combined effectiveness of inulin with other approaches in improving iron nutrition merits future research.

Acknowledgments

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Literature Cited


