Supplemental Dietary Inulin Influences Expression of Iron and Inflammation Related Genes in Young Pigs¹–³

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Abstract
We have previously shown improved hemoglobin (Hb) repletion efficiency by supplementing a 50:50 mixture of short (P95) and long-chain (HP) inulin (Synergy 1, BENEO-Orafti) into a corn-soybean meal-basal diet (BD) for young pigs. In this study, weanling pigs (5 or 6 wk old) were fed the BD or the BD + 4% of P95, HP, or Synergy 1 (50:50 mixtures of HP and P95) for 5–7 wk. Blood Hb concentrations of pigs were measured weekly and digesta samples were collected at the end of the trial. In a replicate experiment, total RNA was isolated from the liver and mucosa of duodenum, ileum, cecum, and colon of all pigs at the end of the trial. Relative mRNA expression of 27 genes, including iron and inflammation-related genes, was quantified using real-time quantitative-PCR. Although all 3 types of inulin resulted in similar improvements ($P < 0.05$) in blood Hb concentration and liver ferritin protein amount, neither type of inulin was detectable in the digesta of cecum or colon. Supplemental inulin enhanced the expression of iron-storing protein genes but decreased that of inflammation-related genes. Such effects were more pronounced ($P < 0.05$) in the mucosa of the lower than the upper gut and were seen on 7 genes in liver. In conclusion, all 3 types of inulin shared similar efficacy and possibly similar modes of action in improving dietary iron utilization by young pigs. Suppressing inflammation-induced genes that can negatively influence iron metabolism might help explain the benefit of inulin. J. Nutr. 139: 2018–2023, 2009.

Introduction
Inulin is a generic term describing all $\beta$ (2–1) linear fructans (1) and is found in tissues of various plants (2). Previously, our group showed a positive effect on hemoglobin (Hb)⁸ repletion efficiency in young pigs by supplementing a 50:50 mixture of short and long-chain inulin (Synergy 1, Orafti) into a corn-soybean meal-basal diet (3). We also demonstrated that the cecum was the main site of disappearance for the supplemental inulin in those pigs (4). Consequently, it was of interest to determine whether inulin’s chain length (short or long) influenced its iron bioavailability-promoting effect and metabolism.

Furthermore, our previous experiments used only Hb repletion efficiency to estimate the inulin benefit (3). Liver ferritin is widely considered to be a sensitive indicator of iron status, particularly under nonanemic conditions or moderate iron adequacy (5). To the best of our knowledge, there has not been a reported success in the cross-reactivity of an antibody against human ferritin with the porcine liver homogenate. Although a number of groups have reported effects of inulin on population changes of colonic microbes (6–9), modulation of lipid metabolism (10–12), enhancement of intestinal immunity (13–16), and changes of mineral (i.e. Ca, Mg, and Fe) bioavailability (3,17–20), little is known about the mechanism for the positive effects of inulin on the bioavailability of iron. We did not observe a substantial shift of pH (6,21,22), mineral solubility (3,17), concentrations of the iron chelators sulfide and fructose, or phytase and inulinase activities (3,4) in the intestinal digesta of pigs fed inulin. Most likely, the benefit of inulin is beyond the digestive stage, because iron utilization and homeostasis are regulated not only at iron absorption, but also at iron transfer, storage, and recycling stages (23–26).

Supplemental Table 1–7 are available with the online posting of this paper at jn.nutrition.org.

¹ Supported in part by a grant from Harvest-Plus, International Food Policy Research Institute and Centro Internacional Agricultura Tropical and BENEO-Orafti (Tienen, Belgium). Mention of a trademark, proprietary product or vendor does not constitute a guarantee or warranty of the product by the United States Department of Agriculture and does not imply its approval to the exclusion of other products or vendors that may also be suitable.
³ Supplemental Tables 1–7 are available with the online posting of this paper at jn.nutrition.org.
⁴ Abbreviations used: BD, basal diet; Hb, hemoglobin; TBST, Tris-buffered saline with Tween 20.
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were conducted with a total of 52 weanling Yorkshire pigs housed into 4 pens (n = 5/pen) and fed the same diets as in Expt. 1 for 7 wk. Pigs were housed in an environmentally controlled barn (22–25°C; light:dark cycle of 12:12 h), given free access to feed and water, and checked daily.

Materials and Methods

Basal diet, inulin, and animals. The basal diet (BD) consisted of corn and soybean meal (Table 1) and contained adequate concentrations of all nutrients (27) except for iron (no inorganic iron supplement). The supplemental (at 4%) inulin sources (Beneo-Orafti) were as follows: Raftilose HP [α-α-glucopyranosyl-(BD-fructofuranosyl)₇-β-D-fructofuranosides (n = 10–60, average of 25)], Raftilose P95 [α-α-glucopyranosyl-(BD-fructofuranosyl)_1-β-D-fructofuranosides (n = 2–7, average of 4)], and Synergy 1 (50:50 mixtures of HP and P95). The same formulations were used to prepare the experimental diets for Expt. 2–7, average of 4), and Synergy 1 (50:50 mixtures of HP and P95). The same formulations were used to prepare the experimental diets for Expt. 1 and 2, but the analyzed inulin concentrations differed somewhat, probably due to sampling and analysis variation. The 2 experiments were conducted with a total of 32 weaning Yorkshire × Hampshire × Landrace crossbred pigs from the Cornell University Swine Farm. Protocols for both experiments were approved by the Cornell University Institutional Animal Care and Use Committee. All experimental pigs were injected with only one-half of the normal iron dose (50 mg of iron as Fe-dextran) at birth. In Expt. 1, 32 pigs (6 wk old; body weight, 9.2 ± 0.4 kg; barrows:gilts, 17:15) were individually penned and fed the BD or the BD plus 4% of Synergy 1, HP, or P95 for 5 wk. In Expt. 2, 20 pigs (5 wk old; body weight, 9.1 ± 0.5 kg; barrows:gilts, 8:12) were group-housed into 4 pens (n = 5/pen) and fed the same diets as in Expt. 1 for 7 wk. In Expt. 2, body weight, blood Hb, and hematocrit were measured as in Expt. 1. At the end, segments of duodenum, ileum, cecum, and proximal colon were quickly removed and rinsed with iced saline solution. The collection method and the exact location of different segments were the same as previously described (3). Intestinal mucosa samples were collected for gene expression analysis from these segments by scraping only the mucosa layer of the tissue using a glass slide. The liver tissue samples (~3 g) were obtained from the caudate lobe of the liver. Collected samples were immediately frozen in liquid N₂ and stored in a −80°C freezer until analysis.

RNA isolation real-time PCR analysis. Intestinal mucosal and liver tissue samples were homogenized in TRIzol Reagent (Invitrogen) to isolate total RNA. The RNA integrity and quantity were assessed using the Agilent Bioanalyzer 2100 and RNA 6000 Labchip kit (Agilent Technologies). The cDNA for respective genes was synthesized using Superscript and random primer/oligo dT mixture following the manufacturer’s instructions (Invitrogen). Real-time PCR was performed using a commercial kit (Abbene, 25 µl/well and 75 ng of RNA/cDNA equivalent/well) on an ABI 7700 (Applied Biosystems) (28). The sequences of the genes and primers are referred to in Supplemental Table 2. In addition to 25 iron (ACO1, B2M, CP, CYBRD1, FTH1, FTL1, HAMP, HEPH, HFE, HFE2, HIP1A, HMOX1, IREP2, LTF, MFI2, MUC5AC, PECT, SLCA1A, SLCAJ2A, SLC4A1, TF, TRF2, TFRC, TNF, and UBE2D1) and 2 vitamin A and lipid metabolism-related (ABCA1 and SCARB1) genes, a panel of 4 housekeeping genes (RPLP0, RPL32, UBC, and PPIA) was run for each tissue. The housekeeping gene that demonstrated the least variance and lowest statistical trend related to treatment was used for normalization. Data for gene expression in the liver, duodenum, ileum, cecum, and colon were adjusted for the housekeeping gene (UBC was used for liver, duodenum, ileum, and cecum and RPL32 was used for colon). The housekeeping gene-adjusted data were analyzed using the ΔΔCT method (28) and the pigs fed BD as the control.

Western blot analyses of the liver ferritin. We used an affinity-purified goat polyclonal antibody raised against a peptide mapped within an internal region of human ferritin light chain (C20: sc-14422, Santa Cruz Biotech). Because the human antigen shares 89% (17/19) sequence similarity to that of corresponding porcine ferritin light chain region, we predicted a cross-reactivity of the antibody with the pig ferritin, although that was not tested by the supplier. Pig liver samples were homogenized in PBS and centrifuged at 14,000 × g for 10 min at 4°C. After the protein concentration in the resulting supernatants was determined using the bicinchoninic acid assay (Pierce), the homogenates (100 µg protein) were loaded onto a SDS-polyacrylamide gel (12%) for electrophoresis and then transferred to nitrocellulose membranes. The membranes were first blocked in Tris-buffered saline with 0.1% Tween 20 (TBST) containing 3% milk for 1 h at room temperature before being incubated overnight with the goat anti-human ferritin light chain (1:400 in 3% milk TBST) at 4°C with constant motion. After 3 washes (5 min each) with TBST, the blot was incubated with an anti-goat horseradish peroxidase (Santa Cruz, 1:5000, 3% milk TBST) for 1 h at room temperature. The blots were washed again 3 times with TBST followed by 5 rinses in deionized water. The resultant protein bands were visualized using SuperSignal West Pico chemiluminescent substrate system (Pierce). The density of the protein bands was quantified using the Alpha-Imager 2200 system.

TABLE 1 Composition of the experimental diets

<table>
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<tr>
<th>Ingredient</th>
<th>BD</th>
<th>Synergy 1</th>
<th>HP</th>
<th>P95</th>
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<tr>
<td>Corn</td>
<td>591.4</td>
<td>591.4</td>
<td>591.4</td>
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</tr>
<tr>
<td>Soybean meal, 48% CP</td>
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<td>306.5</td>
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<tr>
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<tr>
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<td>14.5</td>
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<td>10.0</td>
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<tr>
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<td>2.5</td>
<td>2.5</td>
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<tr>
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<td>10.0</td>
<td>10.0</td>
<td>10.0</td>
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<td>l-Lysine</td>
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<td>Tylan</td>
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<td>5.0</td>
<td>5.0</td>
<td>5.0</td>
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<td>40.0</td>
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Nutritional values

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<th>P95</th>
</tr>
</thead>
<tbody>
<tr>
<td>ME, kcal/kg</td>
<td>13.8</td>
<td>13.2</td>
<td>13.2</td>
<td>13.2</td>
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<tr>
<td>Crude protein, %</td>
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<td>20.3</td>
<td>20.3</td>
<td>20.3</td>
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<tr>
<td>Fe, mg/kg</td>
<td>100.8</td>
<td>94.9</td>
<td>95.7</td>
<td>112.1</td>
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<td>Inulin, g/kg</td>
<td>5.1</td>
<td>38.4</td>
<td>41.9</td>
<td>44.8</td>
</tr>
<tr>
<td>Exp. 1</td>
<td>3.4</td>
<td>24.3</td>
<td>27.4</td>
<td>26.7</td>
</tr>
</tbody>
</table>

1 Vitamin and mineral premix provided/kg diet: retinol palmitate, 1208 µg; ergocalciferol, 5.5 µg; α-tocopherol acetate, 10.72 mg; menadione, 0.5 mg; α-tocopherol, 0.05 mg; choline chloride, 0.5 mg; folic acid, 0.03 mg; niacin, 15 mg; CaO-pantothenate, 10 mg; riboflavin, 3.5 mg; thiamin, 1 mg; pyridoxine, 1.5 mg; vitamin B-12, 17.5 µg; Cu (CuSO₄·5H₂O), 6 mg; I, 0.14 mg (C₆H₄N₂H₂₂, ethylene diamine dihydroiodide); Mn, 4 µg (MnO); Se, 0.3 mg (Na₂SeO₃·2H₂O); Zn, 100 mg (ZnO).
3 Analyzed using an ICAP (BIE Trace Analyzer, Thermo Electron).
4 Analyzed as described by Quermer et al. (46).
(Alpha Innotech) and normalized to that of β-actin on the same membrane. The ferritin protein level in the inulin-fed pigs was expressed as a relative percentage to the mean of the control pigs.

**Statistical analyses.** Data were analyzed as a randomized block design using the Proc General Linear Models procedure of SAS (version 6.12, SAS Institute). The main effects of supplemental dietary inulin on various measures were analyzed using 1-way ANOVA with or without time-repeated measurements. We used the Bonferroni t-test to compare treatment means and the significance level was set at $P < 0.05$ (29). The individual pigs were the experimental unit. Values are expressed as means ± SE.

**Results**

**Expt. 1.** Whereas the initial (wk 0) blood Hb concentrations were similar in the 4 groups of pigs, the final blood Hb concentrations in pigs fed inulin, irrespective of the type, were ~10% higher ($P < 0.05$) than those fed BD (Table 2). Pigs fed HP and P95 had 8–9% higher ($P < 0.05$) final hematocrit and 17–22% greater ($P < 0.05$) feed intakes than pigs fed BD and/or Synergy 1. Final body weight or body weight gain did not differ among the treatment groups. Pigs fed HP had a higher ($P < 0.05$) inulin concentration in the lower jejunum digesta than pigs fed Synergy 1 (53%) and P95 (230%) (Fig. 1). Inulin concentrations in the ileum digesta did not differ among the 3 inulin-fed groups. Inulin was undetectable in digesta samples from the cecum, proximal, mid, or distal colon in any of the inulin-fed groups.

**Expt. 2.** Supplemental dietary inulin, irrespective of type, did not exert an effect on overall growth performance of pigs but enhanced ($P < 0.05$) final blood Hb concentrations of pigs by 6–14% compared with those fed BD (106.3 g/L). Consistent with these observations, the amount of liver ferritin protein in pigs fed Synergy 1 (53%) and P95 (230%) (Fig. 2). Major changes in gene expressions are presented in Table 3 and complete datasets are provided in Supplemental Tables 3–7. The mRNA levels of *FTL* (ferritin light chain) in both liver and cecum and *HEPH* (hephaestin) in cecum were upregulated ($P < 0.05$) by all 3 types of inulin over the BD. The mRNA levels of *SLC40A1* (ferroportin 1) in duodenum, *SLC11A1* (solute carrier family 11 or MTP, heme carrier protein 1) in cecum and *UBE2D1* (also known as stimulator of iron transport or SFT) in liver were upregulated ($P < 0.05$) by Synergy 1 and P95 over the BD. Meanwhile, Synergy 1 enhanced ($P < 0.05$) mRNA levels of *FTH1* (ferritin heavy chain) in cecum and *MUC5AC* (Mucin 5AC, oligomeric mucus/gel-forming) in cecum was decreased ($P < 0.05$) by HP.

**Discussion**

Results from Expt. 1 indicate that supplementing the long-chain HP and the short-chain P95 to the corn-soy diet of weanling pigs improved blood Hb concentrations similarly to that by their 50:50 mixture, Synergy 1. The improvement by the 3 types of inulin is consistent with our previous observation on Synergy

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**FIGURE 1** Effects of dietary supplemental inulin type on inulin concentrations of digesta in various gastrointestinal tract segments of pigs (Expt. 1). The values (%) are the concentration of inulin detected in the digesta collected at a given site and do not reflect the proportion of inulin recovered from the diet. Values are means ± SE, $n = 8$. Means without a common letter differ, $P < 0.05$.

**FIGURE 2** Effects of dietary supplemental inulin type on relative ferritin protein abundance in the liver of pigs (Expt. 2). Values are means ± SE, $n = 3$. Means without a common letter differ, $P < 0.05$.

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**TABLE 2** Effects of dietary supplemental inulin type on blood Hb concentration, hematocrit, and growth performance of pigs (Expt. 1)$^1$

<table>
<thead>
<tr>
<th>Time, wk</th>
<th>BD</th>
<th>Synergy 1</th>
<th>HP</th>
<th>P95</th>
<th>SEM</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb, g/d</td>
<td>0</td>
<td>86.6</td>
<td>83.7</td>
<td>85.0</td>
<td>84.1</td>
<td>1.8</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>117.8$^b$</td>
<td>129.4$^a$</td>
<td>128.0$^b$</td>
<td>130.0$^a$</td>
<td>2.3</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>0</td>
<td>0.34</td>
<td>0.35</td>
<td>0.34</td>
<td>0.35</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>0.39$^a$</td>
<td>0.41$^a$</td>
<td>0.42$^a$</td>
<td>0.43$^b$</td>
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<td>Body weight, kg</td>
<td>0</td>
<td>9.1</td>
<td>8.8</td>
<td>9.2</td>
<td>9.5</td>
<td>0.4</td>
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<tr>
<td></td>
<td>5</td>
<td>32.1</td>
<td>32.1</td>
<td>33.7</td>
<td>34.2</td>
<td>1.1</td>
</tr>
<tr>
<td>Weight gain, g/d</td>
<td>0–5</td>
<td>650.7</td>
<td>663.9</td>
<td>699.5</td>
<td>693.9</td>
<td>0.02</td>
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<tr>
<td>Feed intake, g/d</td>
<td>0–5</td>
<td>1118.4$^b$</td>
<td>1182.6$^b$</td>
<td>1310.2$^b$</td>
<td>1359.2$^a$</td>
<td>48.6</td>
</tr>
</tbody>
</table>

$^1$ Values are mean, $n = 8$. In a row with superscripts without a common letter differ, $P < 0.05$. Data were analyzed using 1-way ANOVA with (Hb, hematocrit, and body weight) or without (overall weight gain and feed intake) time-repeated measurements.
Influenza gene expression. Third, supplemental inulin affected
compounds or metabolites of inulin, rather than intact inulin,
disappeared in cecum, the differences between the upper and
affected by the supplemental inulin in cecum and colon than in
similar mechanism or mode of action. Second, more genes were
up- or downregulation of any given affected gene, implying a
types of inulin exerted consistent (the same direction or trend)
profiles of the iron metabolism-related genes in pigs fed the 3
and possibly a similar mode of action in improving dietary iron
small numerical or statistical differences in inulin concentrations
ferritin protein by the 3 types of inulin over the BD. Despite
Western blot analysis illustrated an upregulation of pig liver
mucosal tissues of cecum and colon compared with those fed
mRNA levels of the ferritin light chain gene in the liver and
1 (3). Furthermore, pigs fed all 3 types of inulin had increased
mRNA levels of the ferritin light chain gene in the liver and
mucosal tissues of cecum and colon compared with those fed
BD. Western blot analysis illustrated an upregulation of pig liver
ferritin protein by the 3 types of inulin over the BD. Despite
numbers or statistical differences in inulin concentrations
in the lower jejunum and ileum digesta among the 3 types of
inulin, the cecum was the main site of disappearance (4) for all 3
types. Overall, these 4 lines of evidence suggest that all 3 types of
inulin, independent of their chain length, share similar efficacy
and possibly a similar mode of action in improving dietary iron
utilization by young pigs fed a plant-based diet without
supplemental inorganic iron.

Comparative analysis of the overall mRNA expression profiles of the iron metabolism-related genes in pigs fed the 3
types of inulin with those fed BD provided 3 clues. First, the 3
types of inulin exerted consistent (the same direction or trend)
up- or downregulation of any given affected gene, implying a
similar mechanism or mode of action. Second, more genes were
affected by the supplemental inulin in cecum and colon than in
duodenum and ileum. Because the ingested inulin mainly
disappeared in cecum, the differences between the upper and
lower gut mucosa in gene expression suggested that the degraded
compounds or metabolites of inulin, rather than intact inulin,
influenced gene expression. Third, supplemental inulin affected
gene expression in liver, suggesting the effect of inulin on iron
metabolism beyond the digestive tract.

From the functional standpoint, supplementing inulin in the
diet resulted in upregulation of iron-storing and sequestering
protein genes, including *FTL, TFRC*, and *FTH1* and
downregulation of inflammation-related genes, including *TNF, TFRC,*
and *SLC11A1.* The upregulation of the former was consistent
with the improved iron or Hb status of pigs fed inulin. The
downregulation of the latter indicated an improved general
health status or immunity, along with a possible sparing of iron
from macrophage iron recycling (30,31). *TNF* is a cytokine
involved in systemic inflammation and is mainly produced by
resident macrophages in response to lipopolysaccharide and
other proinflammatory stimuli (32,33). Expression of transfer-
receptor (*TFRC*) is considered as an indicator of inflam-
(34,35), although its exact role in enterocyte iron
metabolism has not been resolved and is a subject of active
investigation. Interestingly, it has been documented that
*TFRC* associates with *HFE* and *B2M* (36) at the basolateral surface and
may participate in the sensing of whole-body iron via accumu-
lation of an intracellular labile iron pool. In addition, *TFRC* and
*SLC11A1* are also positive indicators of tissue iron (34,37).
Therefore, further investigation is necessary to understand
how these genes were downregulated in pigs that had improved
iron status. The expressions of proinflammatory mediators,

### TABLE 3: Effects of dietary supplemental inulin type on mRNA expression of iron and
inflammation-related genes in pigs (Expt. 2)  

<table>
<thead>
<tr>
<th>Gene symbol</th>
<th>Gene name</th>
<th>Fold induction</th>
<th>Synergy 1</th>
<th>HP</th>
<th>PBS</th>
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<td>Duodenum</td>
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<tr>
<td>SLC11A1</td>
<td>Solute carrier family 11 (NRAMP1)</td>
<td>—</td>
<td>—</td>
<td>(2.0 ± 0.2)</td>
<td>(2.3 ± 0.3)</td>
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<tr>
<td>SLC40A1</td>
<td>Ferroportin 1</td>
<td>↑</td>
<td>↑</td>
<td>(2.7 ± 0.2)</td>
<td>—</td>
<td>0.03</td>
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<td>Ileum</td>
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<tr>
<td>SCARB1</td>
<td>Scavenger receptor class B, member 1</td>
<td>—</td>
<td>↑</td>
<td>(4.0 ± 0.9)</td>
<td>—</td>
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<td>CYBRD1</td>
<td>Cytochrome b reductase 1</td>
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<td>↑</td>
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<td>Hephaestin</td>
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<td>↑</td>
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<td>(1.5 ± 0.1)</td>
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</tr>
<tr>
<td>MUCSAC</td>
<td>Mucin 5, subtypes A and C</td>
<td>—</td>
<td>↓</td>
<td>(3.0 ± 0.1)</td>
<td>—</td>
<td>0.02</td>
</tr>
<tr>
<td>PCT</td>
<td>Heme carrier protein 1</td>
<td>↑</td>
<td>↑</td>
<td>(1.6 ± 0.1)</td>
<td>(1.7 ± 0.1)</td>
<td>0.01</td>
</tr>
<tr>
<td>SLC11A1</td>
<td>Solute carrier family 11 (NRAMP1)</td>
<td>↑</td>
<td>↑</td>
<td>(1.5 ± 0.1)</td>
<td>(1.6 ± 0.1)</td>
<td>0.03</td>
</tr>
<tr>
<td>Colon</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HAMP</td>
<td>Hepcidin antimicrobial peptide</td>
<td>—</td>
<td>↑</td>
<td>(2.8 ± 0.6)</td>
<td>(2.4 ± 0.3)</td>
<td>0.03</td>
</tr>
<tr>
<td>IREP2</td>
<td>Iron-responsive elem binding protein 2</td>
<td>↓</td>
<td>↓</td>
<td>(1.5 ± 0.1)</td>
<td>(1.9 ± 0.1)</td>
<td>0.03</td>
</tr>
<tr>
<td>TFRC</td>
<td>Transferrin receptor (p90, CD71)</td>
<td>↓</td>
<td>↓</td>
<td>(3.5 ± 0.1)</td>
<td>(2.5 ± 0.2)</td>
<td>0.001</td>
</tr>
<tr>
<td>TNF</td>
<td>Tumor necrosis factor</td>
<td>↓</td>
<td>↓</td>
<td>(3.0 ± 0.3)</td>
<td>(1.9 ± 0.1)</td>
<td>0.01</td>
</tr>
<tr>
<td>Liver</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CYBRD1</td>
<td>Cytochrome b reductase 1</td>
<td>↑</td>
<td>↑</td>
<td>(3.8 ± 0.5)</td>
<td>—</td>
<td>0.001</td>
</tr>
<tr>
<td>FTL</td>
<td>Ferritin, light polypeptide</td>
<td>↑</td>
<td>↑</td>
<td>(2.2 ± 0.2)</td>
<td>(1.6 ± 0.2)</td>
<td>0.03</td>
</tr>
<tr>
<td>HEPH</td>
<td>Hephaestin</td>
<td>↑</td>
<td>↑</td>
<td>(10.9 ± 1.0)</td>
<td>—</td>
<td>0.02</td>
</tr>
<tr>
<td>HIF1A</td>
<td>Hypoxia-inducible factor 1</td>
<td>—</td>
<td>↑</td>
<td>(2.0 ± 0.2)</td>
<td>—</td>
<td>0.04</td>
</tr>
<tr>
<td>LTF</td>
<td>Lactotransferrin, lactoferrin</td>
<td>↑</td>
<td>↑</td>
<td>(4.7 ± 0.9)</td>
<td>—</td>
<td>0.02</td>
</tr>
<tr>
<td>SCARB1</td>
<td>Scavenger receptor class B, member 1</td>
<td>↓</td>
<td>↓</td>
<td>(3.5 ± 0.2)</td>
<td>(2.5 ± 0.2)</td>
<td>0.03</td>
</tr>
<tr>
<td>UBEZD1</td>
<td>Ubiquitin-conjugating enzyme ZD2</td>
<td>↑</td>
<td>↑</td>
<td>(2.4 ± 0.4)</td>
<td>(3.1 ± 0.5)</td>
<td>0.02</td>
</tr>
</tbody>
</table>

1 Values are means, n = 5. Arrows indicate the direction and significance level of the difference from control: 1 arrow, P < 0.05–0.01; 2
arrows, P < 0.01–0.001; and 3 arrows, P < 0.001.
2 Not different from the BD, P > 0.05.
SLC11A1, an iron-sequestering protein involved in inflammation, and lactoferrin genes were also altered. These genes are expressed in high levels by the enterocytes and neutrophils and activated resident macrophages. Presumably, circulating proteins, including lactoferrin, exit these cells and enter the systemic circulation to affect other distant organs and systems to deliver appropriate signals in response to changes derived by the supplementation of inulin.

Iron homeostasis is primarily controlled at the level of intestinal absorption (25). Our results demonstrated that although there were upregulations of HEPH and downregulation of CYBRD1 in the cecum, other critical components involved in iron transport such as DMT-1 and ferroportin (38, 39) were not consistently affected by different types of inulin. In addition, a key negative regulator of iron absorption, HAMP (40), was upregulated in the colon, suggesting that effects of inulin on iron metabolism may extend beyond improving absorption of iron. This may represent a negative feedback mechanism to decrease iron absorption due to improved body iron status, because hepcidin is an inhibitor of ferroportin that prevents iron export out of iron-storing cells. It remains unclear why certain genes such as hephaestin and IREP2 were affected by only 2 types or 1 type of inulin and why certain genes such as CYBRD1 were altered by inulin in opposite directions between different tissues.

Findings from the present study have several nutritional and health implications. First, because the benefit of inulin on dietary iron bioavailability and body iron status is independent of chain length, various staple crops with different types of inulin may be used to improve human iron nutrition (41). Inulin content and chain length vary greatly between different plant species. For example, wheat contains 1–4% inulin and ~50% of that is composed of short chain (Degree of polymerization: DP <5). On the other hand, globe artichoke contains 2–7% inulin and ~85% of that is composed of long chain (DP > 40) (42). Plant breeders could conveniently enrich target crops with intrinsic types of inulin to effectively promote dietary iron utilization by humans. Further research will be required to deliver positive effects similar to those observed in the current studies with less inulin, as ingestion of high amount of inulin may cause excessive flatus, borborygmi, and bloating (43). Second, the favorable responses of expression profiles of genes related to intestinal inflammation and systemic immunity suggest that inulin may have additional benefits to the iron-deficient populations that typically have a high incidence of infection and immunosuppressive disorders (44). Finally, our success in exploring the cross-reactivity of the antibody against the human ferritin (light chain) with the porcine liver homogenate provides a useful tool to assess iron status of pigs.

Acknowledgments

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Literature Cited


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