Dietary Intrinsic Phytate Protects Colon from Lipid Peroxidation in Pigs with a Moderately High Dietary Iron Intake (44387)

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Abstract. **High iron consumption has been proposed to relate to an increase in the risk of colon cancer, whereas high levels of supplemental sodium phytate effectively reduce iron-induced oxidative injury and reverse iron-dependent augmentation of colorectal tumorigenesis. However, the protective role of intrinsic dietary phytate has not been determined. In this study, we examined the impact of removing phytate present in a corn-soy diet by supplemental microbial phytase on susceptibility of pigs to the oxidative stress caused by a moderately high dietary iron intake. Thirty-two weanling pigs were fed the corn-soy diets containing two levels of iron (as ferrous sulfate, 80 or 750 mg/kg diet) and microbial phytase (as Natuphos, BASF, Mt. Olive, NJ, 0 or 1200 units/kg). Pigs fed the phytase-supplemented diets did not receive any inorganic phosphorus to ensure adequate degradation of phytate. After 4 months of feeding, liver, colon, and colon mucosal scrapings were collected from four pigs in each of the four dietary groups. Colonic lipid peroxidation, measured as thiobarbituric acid reacting substances (TBARS), was increased by both the high iron ($P < 0.0008$) and phytase ($P < 0.04$) supplementation. Both TBARS and F_2-isoprostanes, an *in vivo* marker of lipid peroxidation, in colonic mucosa were affected by dietary levels of iron ($P < 0.03$). Mean hepatic TBARS in pigs fed the phytase-supplemented, high iron diet was 43%–65% higher than that of other groups although the differences were nonsignificant. Moderately high dietary iron induced hepatic glutathione peroxidase activity ($P = 0.06$) and protein expression, but decreased catalase ($P < 0.05$) in the colonic mucosa. In conclusion, intrinsic phytate in corn and soy was protective against lipid peroxidation in the colon associated with a moderately high level of dietary iron.**

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The importance of iron in human nutrition and health is well documented (1–4). High dietary intakes of iron may enhance the risk of colon cancer, due to the ability of iron to generate free radicals *in vivo* (5). Because colon cancer is a prevalent disease (average rate 42.8/100,000) with a high mortality rate (average rate 15.7/100,000 (6), it is critical to develop ameliorating strategies. Consumption of fibrous bran products seems to be effective in the prevention of colon cancer (7), and phytic acid in the bran has been suggested as the component responsible for this protection (8). Several researchers have shown that high levels of exogenous sodium phytate indeed reduce iron-induced oxidative injury and reverse iron-dependent augmentation of colorectal tumorigenesis in rats (8). However, there was no direct experimental evidence to confirm this presumed protective effect of the intrinsic dietary phytate present in ordinary foods, particularly in the cases of moderately high iron overload.

This experiment was conducted to test the hypothesis that moderately high dietary iron intakes could produce oxidative stress in the colon of pigs and that the resulting oxidative injuries would be aggravated by removal of dietary intrinsic phytic acid. We used pigs as our animal model due to great similarities in the gastrointestinal tract

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(9) and iron metabolism (10) between this species and humans. In addition to the colon as an obvious target organ, the liver was also examined because of its important role in iron storage.

Materials and Methods

Animals. Thirty-two crossbred barrows and gilts (Hampshire × PIC 15, 28 days of age, body weight: 10.35 ± 0.66 kg) were separated into four treatment groups based on sex, litter, and body weight. Pigs were housed in cement floor pens (3 × 2.5 m), and the room temperature was controlled at 21 ± 2°C throughout the experiment. Animals were given free access to feed and water during the 15-week trial. Our protocol was approved by the Institutional Animal Care and Use Committee at Cornell University and conducted in accordance with the NIH guidelines for the care and use of experimental animals.

Diets. The experimental diets were corn-soybean meal based. Two levels of iron (80 or 750 mg/kg) were supplemented in the form of ferrous sulfate to resemble normal and moderately high dietary intakes of this mineral (11). Analyzed iron concentrations measured in the diet were 140 and 845 mg/kg for the low and high iron supplementation, respectively. Two levels of phytase (0 or 1200 units/kg) were added using a microbial phytase (Natuphos, BASF, Mt. Olive, NJ). The enzyme sequentially cleaves phosphate groups from the inositol ring of phytate in the stomach and proximal small intestine of the pig, and the expected treatment effects are often observed within 1 week (12–14). Because inositol phosphates with a lower degree of phosphorylation lose their chelating capacity, iron would remain largely free and able to cause oxidative stress. Analyzed phytase activity in the supplemented diets was 1200 units/kg, and phytate content was similar in all the experimental groups (1.1%–1.3%). *In vitro* phosphorus release assays were performed to ensure that phytase activity remained intact during feed storage prior to consumption by the animals. All diets (Table I) were formulated to meet the nutritional requirements of swine at different stages (11). No inorganic phosphorus was added to the phytase-supplemented diets to ensure an adequate degradation of phytate, and the calcium/phosphorus ratio was adjusted to 1.2.

Sample Collection. Individual body weights were recorded, and blood samples were collected from the jugular vein into heparinized tubes monthly. At the end of the experiment, four pigs from each treatment group were sacrificed, and a portion of the liver was collected. After rinsing with physiological saline, samples of whole colon were collected from the distal part of the large intestine with mucosal cells intact. Mucosal cells from the distal colon were scraped using a microscope slide. Samples were frozen in liquid nitrogen and stored at −80°C until analyzed. Samples of proximal and distal colon were fixed in 10% buffered formalin, embedded in paraffin, sectioned at 4 μm, and stained with hematoxylin and eosin. The slides were examined by a pathologist at Cornell University.

Table I. Diet Composition (Percentage as Fed Basis)[a]

	No phytase	Phytase
Corn	63.22	63.72
Soybean meal (44%)	30	30
Corn oil	2	2
Lys · HCl	0.28	0.28
Methionine	0.1	0.1
$Ca_2(PO_4H)_3$	0.95	—
Limestone	0.95	0.9
Salt	0.5	0.5
CSP-250 (antibiotics)	0.5	0.5
Vitamin & mineral premix[bc]	0.5	0.5
Fe Premix[d]	1	1
Phytase premix[e]	—	0.5

[a] Levels of corn, soybean meal, lysine, methionine, dicalcium phosphate, and limestone were adjusted in the diet throughout the experimental period in accordance with the nutrient requirements for swine (11).
[b] All premixes used corn as a carrier.
[c] Vitamin and mineral premix supplies: 2,540 IU Vitamin A, 660 IU Vitamin D, 15 IU Vitamin E, 2.2 mg Vitamin K, 3.3 mg Riboflavin, 13.2 mg Panthotenic Acid, 17.6 mg Niacin, 0.11 g Choline, 1.98 μg Vitamin B-12, 37.4 mg Mn, 0.6 mg I, 10 mg Cu, 0.3 mg Se, and 100 mg Zn per kg of final diet.
[d] Fe premix (supplied as ferrous sulfate) supplies 80 mg of inorganic Fe/kg diet in normal iron diets and 750 mg of inorganic Fe/kg diet in high iron diets.
[e] Phytase premix supplies 1,200 units phytase/kg diet.

Assays for Antioxidant Enzyme Activities. Heparinized blood samples were centrifuged at 3000 r.p.m., 4°C for 10 min (Model GS-6KR, BECKMAN, Palo Alto, CA) to collect plasma. The red blood cells (100 μl) were washed twice with 900 μl of physiological saline. Then, the same volume of ice-cold, double-distilled water was added, and the samples were frozen at −80°C for 24 hr. After thawing, the hemolysate was either used for determining glutathione peroxidase (GPX) and catalase activity or treated with 400 μl of chloroform:ethanol mixture (150p:250p) and centrifuged at 12000 r.p.m., 4°C for 5 min to remove hemoglobin. Activity of Cu/Zn superoxide dismutase (Cu/Zn-SOD) was assayed in the aqueous layer according to Ukeda *et al.* (15) using a 50-m*M* sodium carbonate buffer (pH 10.2).

Liver and colonic mucosal cells were homogenized in 50 m*M* phosphate buffer (pH 7.8) containing 0.1% Triton X-100 and 1.34 m*M* diethylenetriaminepentaacetic acid (DETAPAC) using a Polytron PT 3100 (Brinkmann Instruments, Inc., Westbury, NY). Homogenates were centrifuged at 19,000 r.p.m., 4°C for 30 min (BECKMAN, Model J2-MI), and the supernatant was used for determining activities of catalase (16), GPX (17), and total and manganese superoxide dismutase (Mn-SOD) (15, 18). Activities of Cu/Zn-SOD were calculated by subtracting Mn-SOD from total SOD activity. Protein concentration was assayed by the method of Lowry (19).

Western Blot. To determine the dietary treatment effect on the protein expression of cellular glutathione peroxidase (GPX 1) in liver and colon mucosa, we performed a Western blot analysis using antihuman GPX 1 antibody (kindly provided by Drs. Q. Shen and P. E. Newburger, University of Massachusetts Medical School, Worcester, MA). Both sources of samples were subjected to 12% SDS-polyacrylamide gel electrophoresis, and the amount of sample loaded per lane was 100 and 250 μg protein for liver and colon mucosa, respectively. Subsequently, proteins were transferred to a Protan nitrocellulose membrane (Schleicher & Schuell, Keene, NH) using a Mini Trans-Blot cell system (Bio-Rad Laboratories, Hercules, CA). For the immuno detection, we followed the instructions outlined in the Immun-Blot Assay Kit (Bio-Rad Laboratories).

Assay for Carbonyl Content. Tissues were homogenized in 125 m*M* KCl, 50 m*M* HEPES buffer (pH 7.4) containing leupeptin (5 μg/ml), pepsatin (7 μg/ml), aprotinin (5 μg/ml), and phenylmethylsulfonylfluoride (PMSF) (40 μg/ml) using the Polytron PT 3100 at a homogenizing speed of 13,000 r.p.m. Homogenates were centrifuged at 36,500 r.p.m., 4°C for 12 min (BECKMAN, model L8–70 *M*). Carbonyl content in plasma, liver, and whole colon tissue was determined spectrophotometrically (20), and in colonic mucosal cells by an immunodetection method (21).

Assay for Thiobarbituric Acid Reacting Substances (TBARS) Content. Tissues were homogenized in a 1.5% KCl solution (1 volume sample: 10 volume buffer) using the Polytron PT 3100 at 27,000 r.p.m. Homogenates were centrifuged at 3000 r.p.m., 0°C for 5 min (BECKMAN, model GS-6KR), and TBARS were assayed in the supernatant according to the method of Ohkawa *et al.* (22).

Assay for F_2-Isoprostanes. Tissue F_2-isoprostanes were determined using an 8-Isoprostane Enzyme Immunoassay Kit (Cayman Chemical Co., Ann Arbor, MI), and samples were prepared according to the manufacturer's instructions. Briefly, 1 ml of homogenate (0.25 *M* sucrose, 0.1 *M* Tris-HCl buffer, pH 7.4) was mixed with 2 ml ethanol, and the samples were allowed to stand at 4°C for 5 min and then centrifuged at 1500*g* for 10 min. Supernatant was collected, an equal volume of 15% KOH was added, and the solution was incubated for 1 hr at 40°C. After incubation, samples were diluted to 10 ml with ultrapure water, and the pH was lowered below 4.0 with HCl. The samples were then purified using a Sep-Pak C_{18} Cartridge (Waters Corp., Millford, MA) and a channeled 20 × 20 cm TLC plate (Whatman Inc., Clifton, NJ) before being applied to the immuno analysis for 8-isoprostanes. Results are expressed as ng F_2-isoprostanes/gram fresh tissue.

Assays for the Nutritional Status. Phytase activity of the diets was determined by the release of inorganic phosphorus from sodium phytate (23). One unit (U) of phytase is defined as the amount of activity that liberates 1 μmol of inorganic phosphorus from sodium phytate per minute at pH 5.5 and 37°C. Phytate content of the diets was assayed according to the method of Latta and Eskin (24). Iron, phosphorus, and calcium contents of the diets were determined simultaneously using an inductively coupled argon plasma emission spectrophotometer (Model ICAP 61E Trace Analyzer, Thermo Jerrel Ash Corporation, Franklin, MA). Samples were digested in a mixture of HNO_3 and $HClO_4$ (9:1 v/v) and then diluted in 5% HNO_3 (25). Hemoglobin content was determined in blood and hemolysates according to Hainline (26). Packed cell volume was determined after blood collection using heparinized microcapillary tubes (Fischer Scientific, Cat # 02–668–66). Nonheme iron content of the liver was measured using the modified Schricker method (27). Plasma inorganic phosphorus was determined according to Gomori (28). Plasma alkaline phosphatase activity (AKP) was measured as an index of phosphorus status of the animals (29) using the enzymatic hydrolysis of p-nitrophenol phosphate to p-nitrophenol according to the method of Bowers and McComb (30). The activity is expressed as milliunits/ml plasma.

Statistics. Analysis of the data was performed with SAS (release 6.11, SAS Institute, Inc. Cary, NC). Main effects were analyzed by 2 × 2 factorial ANOVA with dietary supplementation of iron (80 vs. 750 mg/kg) and phytase (0 vs. 1200 units/kg) as the main treatments. Duncan's multiple range test was used to detect differences between treatment means. Time-repeated measurement analysis was applied to the biochemical data from the different blood collections.

Results

Nutritional Status. Pigs fed the phytase-supplemented diets had body weights and plasma inorganic phosphorus concentrations similar to those of the inorganic phosphorus–supplemented controls at each level of dietary iron at completion of the study (Table II). High dietary iron resulted in a significant increase in plasma alkaline phosphatase activity ($P < 0.01$), a marginal decrease in body weight ($P = 0.09$), and no significant effect on hepatic nonheme iron. Hemoglobin and packed cell volume were not affected by the dietary treatments. Repeated measurement analysis of the blood biochemical indicators showed a similar pattern at any given month throughout the experimental period.

Lipid Peroxidation. There was no significant effect of phytase or iron on hepatic TBARS although pigs fed the high iron, phytase-supplemented diet had values 43%–65% higher than those of other groups (Table III). There was an overall effect of dietary iron ($P < 0.0008$), phytase ($P < 0.04$), and iron × phytase interaction ($P < 0.05$) on colon TBARS. This resulted in a two-fold increase in colon TBARS in pigs fed phytase, compared with that of pigs fed inorganic phosphorus, at the level of 750 mg iron/kg, but no difference at the level of 80 mg iron/kg. Levels of TBARS and F_2-isoprostanes in colonic mucosa were also ($P < 0.006$) increased by the high iron treatment.

Table II. Effect of Iron and Phytase Supplementation on the Nutritional Status of the Pigs at the End of the Experiment

Dietary treatment	1	2	3	4				
Fe (mg/kg diet)	80	750	80	750	SEM[c]	Fe Effect	Phytase effect	Fe × Phytase interaction
Phytase (U/kg diet)	—	—	1,200	1,200				
Final body weight (kg)	90.3	87.2	93.8	86.5	3.0	0.09	NS	NS
Hematocrit (%)	42.7	42.5	42.3	43.7	0.6	NS	NS	NS
Hemoglobin (g/dl)	15.0	14.8	14.0	15.1	0.2	NS	NS	0.02
Plasma-P (mg/dl)	8.3	8.7	9.3	8.6	0.3	NS	NS	NS
AKP (munits/ml)	72.6[a]	89.2[a,b]	77.3[a,b]	95.7[b]	6.6	0.01	NS	NS
Nonheme Fe (μg/g fresh liver)	175.2	199.1	179.5	197.9	19.5	NS	NS	NS

Note. Values are means of four pigs for nonheme iron and eight pigs for other measures.
[a,b] Means within the same row without common superscript are significantly different ($P < 0.05$).
[c] Pooled standard error of the mean.

Table III. Effect of Iron and Phytase Supplementation on Lipid Peroxidation in Liver and Colon of the Pigs

Dietary treatment	1	2	3	4				
Fe (mg/kg diet)	80	750	80	750	SEM[c]	Fe Effect	Phytase effect	Fe × Phytase interaction
Phytase (U/kg diet)	—	—	1,200	1,200				
	Thiobarbituric acid reacting substances (nmol MDA/mg protein)							
Liver	0.38	0.44	0.43	0.63	0.11	0.22	0.27	NS
Colon	0.11[a]	0.34[a]	0.10[a]	0.80[b]	0.10	0.0008	0.04	0.05
Colonic mucosa	0.11[a]	0.23[b]	0.16[a,b]	0.22[b]	0.04	0.03	NS	NS
	F_2-Isoprostanes (ng/g tissue)							
Colonic mucosa	1.8[a]	13.1[c]	2.8[a,b]	11.8[b,c]	3.2	0.006	NS	NS

[a,b] Means ($n = 4$) within the same row without common superscript are significantly different ($P < 0.05$).
[c] Pooled standard of error of the mean.

Protein Oxidation. No significant effect of either high iron or phytase supplementation was observed on the carbonyl contents in plasma, liver, or colon of the pigs (Table IV).

Antioxidant Status. Activities of catalase, glutathione peroxidase, and superoxide dismutase in erythrocytes were not significantly affected by the dietary treatments (Table V). High dietary iron resulted in a marginal ($P = 0.06$) increase in hepatic GPX activity. Western blot analysis indicated an induction of the GPX1 protein by the high iron treatment in liver and colonic mucosa (Fig. 1). Catalase activity was reduced in colonic mucosal cells ($P < 0.05$) by the high iron treatment. The activity of Mn-SOD was slightly increased by both dietary phytase and high iron supplementation.

Histology. There was no lesion or histologic alteration in the colon in any of the pigs sacrificed.

Discussion

In the present study, we used a microbial phytase to break down intrinsic phytate in a corn and soy diet for pigs and supplemented 750 mg iron (ferrous sulfate) to induce oxidative stress. Pigs fed the phytase-supplemented diets, in the absence of inorganic phosphorus, had body weight gain and plasma inorganic phosphorus concentrations similar to those of the inorganic phosphorus supplemented pigs, indicating that intrinsic phytic acid phosphorus in the corn-soy diets was hydrolyzed by microbial phytase. High dietary iron caused a marginal decrease in body weight gain and a significant increase in plasma alkaline phosphatase activity

Table IV. Effect of Iron and Phytase Supplementation on Protein Oxidation of Plasma, Liver, and Colon of the Pigs*

Dietary treatment	1	2	3	4				
Fe (mg/kg diet)	80	750	80	750	SEM[a]	Fe Effect	Phytase effect	Fe × Phytase interaction
Phytase (U/kg diet)	—	—	1,200	1,200				
	Carbonyl contents (nmol/mg protein)							
Plasma	0.52	0.57	0.58	0.51	0.04	NS	NS	NS
Liver	0.81	0.69	0.72	0.66	0.09	NS	NS	NS
Colon	0.85	0.74	0.69	0.82	0.08	NS	NS	NS

* Values are means of eight pigs for plasma carbonyl contents at the last blood collection of the study, and four pigs for the other two tissues.
[a] Pooled standard error of the mean.

Table V. Effect of Iron and Phytase Supplementation on the Antioxidant Status of Erythrocytes, Liver, and Colon Mucosa of the Pigs

Dietary treatment	1	2	3	4				
Fe (mg/kg diet)	80	750	80	750	SEM[c]	Fe Effect	Phytase effect	Fe × Phytase interaction
Phytase (U/kg diet)	—	—	1,200	1,200				
				Erythrocytes				
GPX[d]	154.1	153.0	154.4	160.6	11.2	NS	NS	NS
Cu/Zn-SOD[e]	652.1	642.3	594.9	625.9	21.1	NS	NS	NS
Catalase (× 10^6)[f]	0.6	0.6	0.6	0.6	0.04	NS	NS	NS
				Liver				
GPX[g]	230.8	260.5	216.3	256.3	17.3	0.06	NS	NS
Cu/Zn SOD[h]	75.5	71.7	69.9	73.3	3.3	NS	NS	NS
Mn-SOD[h]	8.8	10.1	8.6	9.3	0.68	0.14	NS	NS
Catalase (× 10^3)[i]	1.3	1.1	1.2	1.4	0.08	NS	NS	0.05
				Colonic mucosa				
GPX[g]	267.2	294.7	241.4	253.4	25.6	NS	NS	NS
Cu/Zn-SOD[h]	13.51	13.8	12.8	14.8	0.91	0.22	NS	NS
Mn-SOD[h]	3.5[a]	4.5[b]	4.4[a,b]	4.9[b]	0.3	0.03	0.04	0.44
Catalase[i]	172.7	130.7	177.5	139.0	19.1	0.05	NS	NS

Note. Values are means of eight pigs for the activities of enzymes in erythrocytes at the last blood collection of the study, and four pigs for those in liver and colonic mucosa.
[a,b] Means within the same row without a common superscript are significantly different ($P \leq 0.05$).
[c] Pooled standard error of the mean.
[d] Activity expressed as nmol of reduced glutathione oxidized/g hemoglobin/min.
[e] Activity expressed as units/100μl RBC.
[f] Activity expressed as nmol H_2O_2/min · g hemoglobin.
[g] Activity expressed as nmol of reduced glutathione oxidized/mg protein/min.
[h] Activity expressed as units/mg protein.
[i] Activity expressed as nmol H_2O_2/min · mg protein. There is a difference ($P < 0.05$) in the combined means of colonic mucosa catalase activity between the two levels of dietary iron.

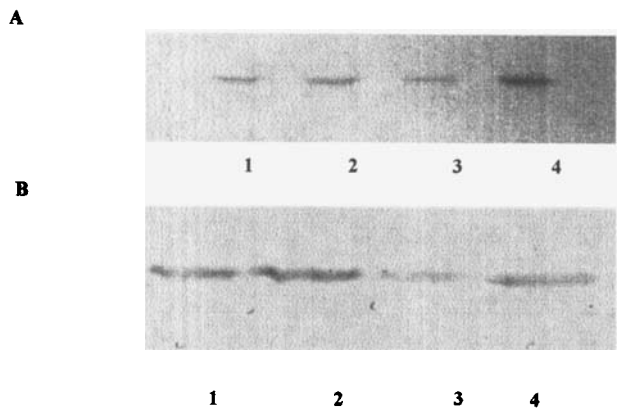

Figure 1. Western blot analysis of cellular glutathione peroxidase (GPX1) expression in (A) liver and (B) colon mucosa of the pigs using an antibody against human GPX1. (Lane 1) Normal iron, no phytase; (Lane 2) Moderately high iron, no phytase; (Lane 3) Normal iron, phytase; (Lane 4) Moderately high iron-phytase. The amount of sample loaded per lane was 100 and 250 μg protein for liver and colon mucosa sample, respectively. The size of the band was about 23 kDa based on prestained SDS-PAGE standards from BIO-RAD laboratories (not shown). This gel is representative of three independent experiments. The image of liver samples is a direct scanning from the gel. The colon mucosa immunoblot has been optimized to ensure adequate contrast of the bands.

(31) and colon lipid peroxidation. Hepatic nonheme iron, a major storage of body iron, did not change significantly in response to the moderately high level of dietary iron supplemented in the present study. Seemingly, a homeostatic regulation mechanism in these pigs might be effective in preventing liver from an accumulation of iron under these circumstances (32).

The significant difference in whole colon lipid peroxidation between pigs fed diets with and without phytase at the moderately high level of dietary iron shows the protective role of intrinsic dietary phytic acid against the iron-induced oxidative stress. Because oxidative stress has been related to an increased risk of colon cancer (33), and we have used a dietary iron concentration lower than previously reported (34, 35), which is not uncommon for certain populations (36), this type of protection of phytate may be physiologically relevant. Previously, high levels of sodium phytate have been shown to reduce incidences of chemically induced colorectal tumorigenesis in experimental animals (37), but there has been little information on the possible benefit of low levels of intrinsic dietary phytate to cope with moderate oxidative stress as employed in the present experiment.

A significant effect of a moderately high dietary iron on colonic mucosal TBARS or F_2-isoprostanes was observed, in agreement with Younes *et al.* (38) and Rimbach *et al.* (34). Due to the relatively large inherent variability of the F_2-isoprostanes assay (39), differences in this measure between the high and low iron groups fed phytase-supplemented diets were significant only at the level of $P = 0.1$, but not at $P = 0.05$. Meanwhile, there was a marginal effect

of dietary iron and phytase on hepatic lipid peroxidation. This could be due to a different susceptibility of liver versus colon to iron-induced oxidative stress or to an insufficient iron level in our experiment to cause a detectable lipid peroxidation in liver. Whereas 80 mg/kg is the quantity of iron normally supplemented in practical diets for growing pigs, 750 mg/kg were used to resemble the levels of consumption of this mineral by certain population groups whose iron intake approximates eight times the normal requirements (36). Yet, this level is much lower than those used in most iron-overload studies (40, 41).

Carbonyl contents, an indicator of protein oxidation (21), in liver, whole colon tissue, and colon mucosa did not show any difference among treatment groups. Either the oxidative stress produced by our moderately high iron treatment was inadequate to cause any protein damage, or the method used to measure protein oxidation is not sensitive enough to detect the possible differences.

There is a great deal of variability in the reported effects of dietary iron overload on antioxidant enzyme activities in tissues (41, 42). In the present experiment, we observed an increase of GPX activity in liver due to relatively low levels of iron compared to what has been used previously in other studies (35). We also detected a relatively high GPX activity in the colonic mucosa although that activity was not affected by the dietary iron levels. Because there are several forms of selenium-dependent glutathione peroxidases present in the liver and gastrointestinal tract of mammals (43), we used the GPX1 antibody and conducted Western blot analysis to corroborate the GPX1 protein and the total GPX activity in liver and colonic mucosa. Results in the liver showed that the increase in total GPX activity coincided with the increase of GPX1 protein expression. In colon mucosa, the GPX1 protein was also induced by the high-iron treatment, but the band was very faint, indicating that GPX1 might not be the major source of the detected total GPX activity. This may also explain why there was no increase in total GPX activity by the high iron treatment in spite of the up-regulation of the GPX1 protein expression. In contrast, catalase activity in colonic mucosa was decreased by the high iron treatment. Kuratko (44) found a similar reduction in catalase activity in rats fed with increasing levels of iron. This decrease might be due to the inhibition of the enzyme activity by lipid peroxides formed in the mucosa (45).

In conclusion, intrinsic phytate from corn-soy diets was beneficial in the prevention of lipid peroxidation in the colon associated with a moderately high level of dietary iron. Although neither dietary iron nor phytase had an effect on protein oxidation in the tissues studied, moderately high dietary iron induced cellular glutathione peroxidase protein and/or activity expression in liver and colon mucosa of the pigs.

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