



The Effect of Iron-enriched *Aspergillus oryzae* on the Growth of Common Bacterial Pathogens

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Abstract

The World Health Organization recommends daily iron supplementation for infants and children and women of reproductive age; however, safety concerns have been raised with these recommendations in areas that have a high infection burden. This is because excess unabsorbed iron may select for enteric pathogens and increase the incidence and severity of infectious disease. *Aspergillus oryzae* (Ao) is a filamentous fungus that can accumulate and store large amounts of iron, and may be less bioavailable to pathogens compared to traditional ferrous sulfate (FeSO₄) supplements. The objective of this study was to determine the effect of iron-enriched Ao compared to FeSO₄ on the growth of prominent Gram-negative enteric pathogens known to cause diarrheal disease (*Salmonella*, *Escherichia*, *Yersinia*, and *Shigella*) and the Gram-positive bacterial strain *Lactobacillus*. Bacterial strains were grown in media containing no iron or 1 μM elemental iron either as FeSO₄ or Ao iron. Bacterial growth was measured at 600 nm every 15 min for 12 h. Growth of each Gram-negative bacteria (*Salmonella*, *Escherichia*, *Yersinia*, and *Shigella*) was greatest when cultured with FeSO₄, conversely their growth was reduced when cultured with Ao iron. Iron had no effect on the growth of *Lactobacillus* strains. These findings suggest that Ao iron may be a suitable alternative to address iron deficiency in areas with a high infection burden.

Introduction

- Daily iron supplementation is recommended for infants and children and women of reproductive age. Safety concerns have been raised with these recommendations in areas that have a high infection burden.
- Only about 10% of iron is absorbed and the remaining 90% of unabsorbed iron travels to the colon where it is available to other microorganism [1].
- Excess iron in the colon as iron sulfate (FeSO₄), the traditional form of iron used for supplementation, has been shown to increase the severity of enteric infections [2].
- This study examines a new form of iron, iron-enriched *Aspergillus oryzae* (Ao), that may be less available to Gram-negative bacteria that cause diarrheal disease.

Objective

To determine the effect of Ao compared to FeSO₄ on the growth of prominent Gram-negative enteric pathogens known to cause diarrheal disease (*Salmonella*, *E. coli*, *Yersinia*, and *Shigella*) and the Gram-positive bacterial strain *Lactobacillus*.

Methods

- The following bacteria were used:
 - *Salmonella* Typhimurium
 - *Enteropathogenic Escherichia coli* (EPEC)
 - *Shigella flexneri*
 - *Yersinia enterocolitica*
 - *Lactobacillus*
- Bacteria were grown in Iscove's Modified Dulbecco Media (IMDM) containing: no added iron (control) or 1 μM elemental iron as either FeSO₄ or FeSO₄-enriched Ao (Ao) (n=3/treatment).
- Bacteria were plated in a 96-well plate and OD₆₀₀ was recorded every 15 min for 12 h using a BioTek Synergy plate reader.
- Area under the curve (AUC) data were analyzed by one-way ANOVA with Tukey's post-hoc comparisons. P<0.05 was considered statistically significant.
- Experiments were repeated at least twice for each strain of bacteria. Data are presented as means ± standard deviation.

Results

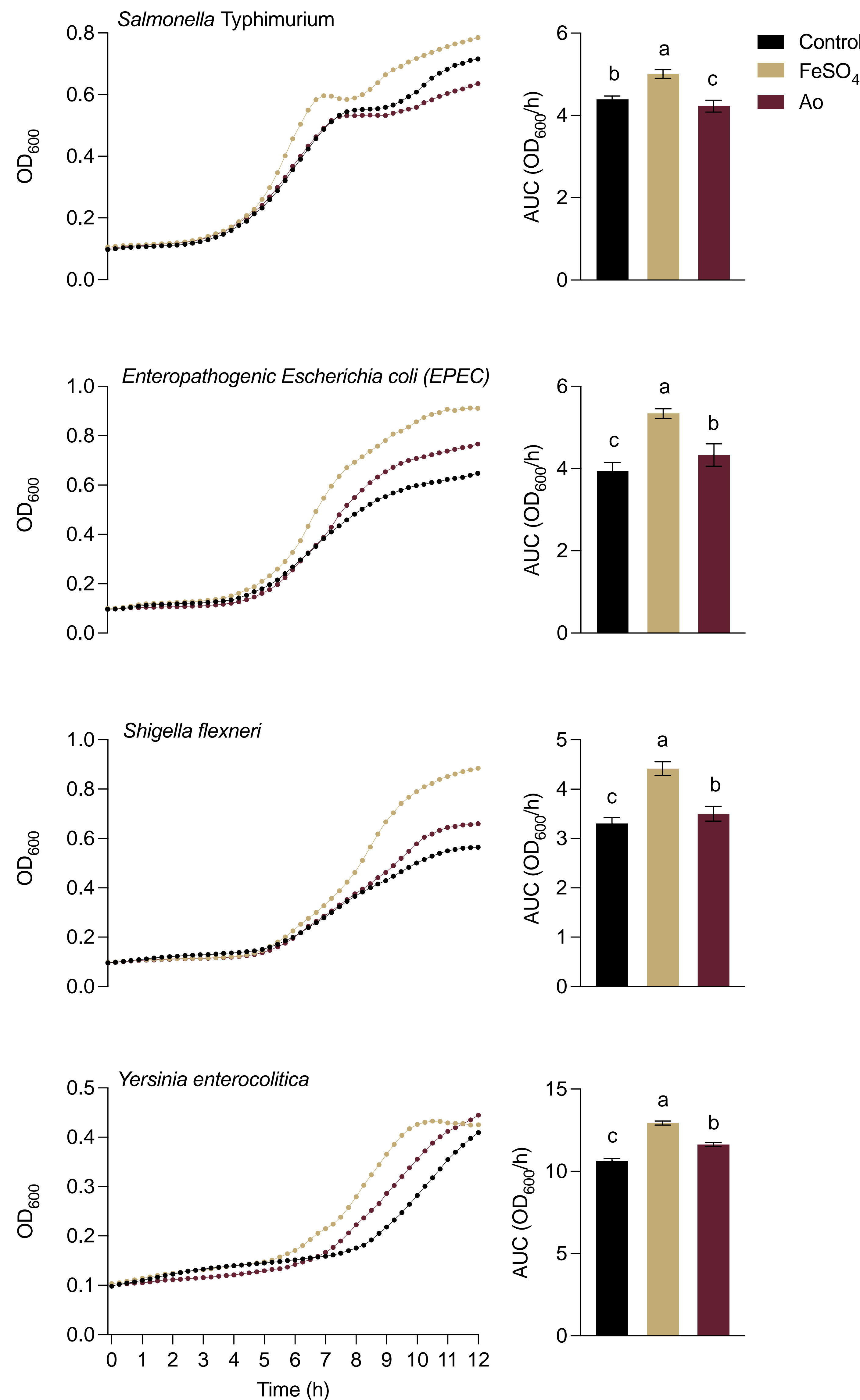


Figure 1. Growth and area under the curve (AUC) of the Gram-negative bacteria *Salmonella* Typhimurium, *Enteropathogenic Escherichia coli* (EPEC), *Shigella flexneri*, and *Yersinia enterocolitica*. Bacteria were cultured in media containing no added iron (control) or 1 μM elemental iron as either FeSO₄ or Ao iron. Different letters indicate a significant difference (P<0.05). Data are means ± standard deviations; n=3/treatment/timepoint.

Results

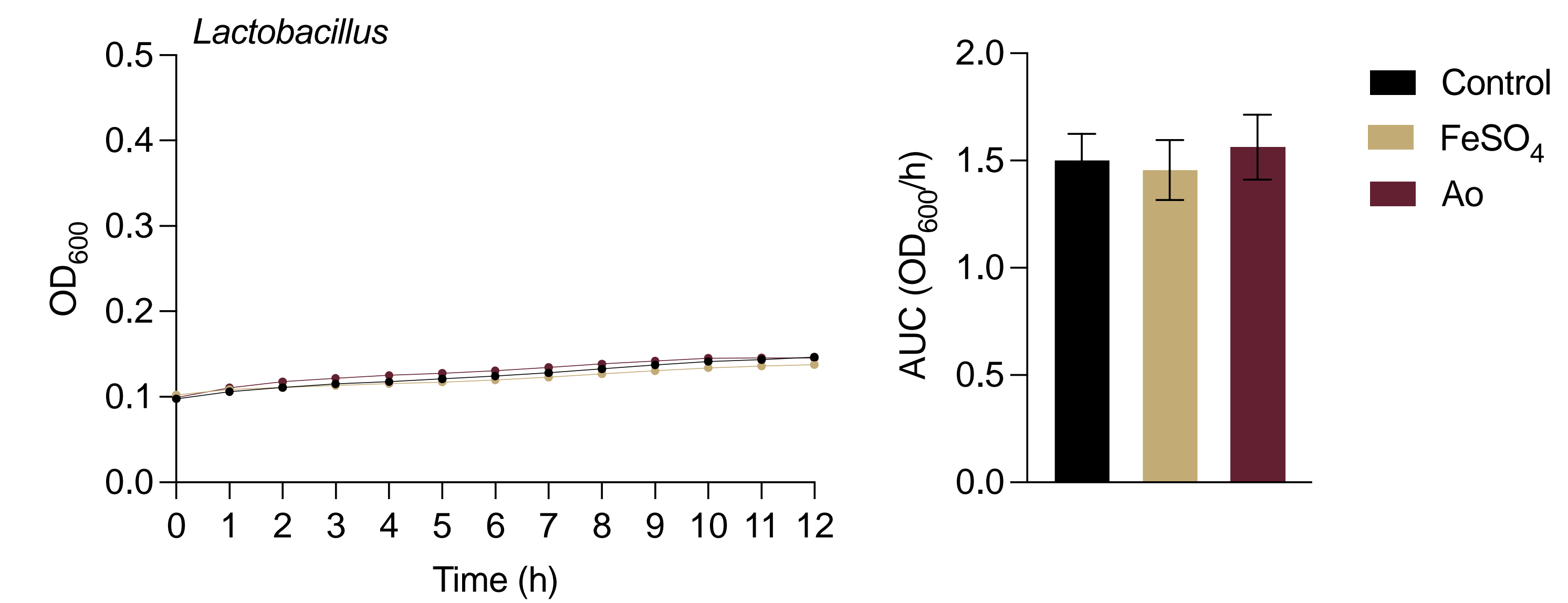


Figure 2. Growth and area under the curve (AUC) of the Gram-positive bacteria *Lactobacillus*. Bacteria were cultured in media containing no added iron (control) or 1 μM elemental iron as either FeSO₄ or Ao iron. Different letters indicate a significant difference (P<0.05). Data are means ± standard deviations; n=3/treatment/timepoint.

	FeSO ₄ vs. Control	Ao vs. Control	Ao vs. FeSO ₄
<i>Salmonella Typhimurium</i>	+14.2%	-3.6%	-15.6%
<i>Enteropathogenic Escherichia coli</i> (EPEC)	+35.6%	+10.0%	-18.9%
<i>Shigella flexneri</i>	+33.8%	+6.1%	-20.7%
<i>Yersinia enterocolitica</i>	+21.4%	+9.1%	-10.1%

Table 1. Comparison of differences in growth of Gram-negative bacteria. Percent difference was determined using area under the curve (AUC) data for each Gram-negative bacteria studied.

Conclusions

- The growth of all Gram-negative bacteria was attenuated in the presence of iron-enriched Ao compared to FeSO₄ and Gram-negative bacteria grown with Ao were comparable to that of the control group in which no iron was present.
- Iron had no effect on the growth of the Gram-positive bacteria.
- These findings suggest that Ao iron may be a suitable alternative to address iron deficiency in areas with a high infection burden.

References

1. Andrews, S. C., Robinson, A. K., & Rodríguez-Quiñones, F. (2003). Bacterial iron homeostasis. *FEMS Microbiology Reviews*, 27(2–3), 215–237.
2. Kortman, G. A. M., Boleij, A., Swinkels, D. W., & Tjalsma, H. (2012). Iron Availability Increases the Pathogenic Potential of *Salmonella* Typhimurium and Other Enteric Pathogens at the Intestinal Epithelial Interface. *PLoS ONE*, 7(1).