

ABSTRACT

This study evaluated the effects of lipopolysaccharide (LPS) injection on the immune response over a 24-h period in nursery pigs. Pigs consumed corn-soybean meal-based diets with added pharmacological levels of ZnO (d 0-14) and CuSO₄ (d 14-23). On d 23, thirty pigs were randomly blocked based on BW and sex to one of five-time points (h 0 baseline, 3, 6, 12, and 24 post-challenge) and injected with a single i.m. LPS (O55:B5) at 12 µg/kg BW. At each time point, BW, rectal temperature (RT), and blood samples (n = 30, 24, 18, 12, and 6 per time point, respectively) were collected before one block (n = 6) was euthanized for liver and duodenum collection. Tissue samples were quantified for interleukin-6 (IL-6), zinc transporters (duodenal Zip4 and hepatic Zip14), and metallothionein-1 (MT-1) mRNA expression. Data were analyzed using PROC GLIMMIX of SAS with pig as the experimental unit. Following LPS, RT increased from h 0 to 6 (P < 0.05), and serum TNF-α increased from h 0 to 3 (P < 0.0001). Serum zinc and copper decreased (P < 0.01) from h 0 to 6, and h 0 to 12, respectively. Serum C-reactive protein tended to increase linearly following LPS (P = 0.10). LPS upregulated duodenal Zip4 and MT-1 (P < 0.05) at h 12 and 24, respectively, while all hepatic genes increased (P < 0.01) at h 3 post-challenge. Duodenal IL-6 did not change over time (P > 0.05). Quantification of mRNA expression displayed a positive correlation (P < 0.01) among hepatic IL-6, Zip14, and MT-1 in pairwise comparisons. In summary, LPS challenge induces fever and hepatic inflammation with consequent increases in hepatic and duodenal zinc importers, and their metal-binding protein, along with decreases in serum zinc and copper concentrations. However, our data indicate that pigs recover within 24-h post-challenge.



INTRODUCTION

- Typical North American nursery pig diets commonly include pharmacological levels of dietary zinc (2,000-3,000 mg/kg) and copper (100-200 mg/kg) for growth and health purposes.
- Decrease in serum zinc concentration or hypozincemia occurs during inflammatory response induced by lipopolysaccharide (LPS) challenge of nursery pigs (Aparachita et al., 2018).
- However, tissue zinc regulating genes, response pattern, and their relationships during inflammation have not been investigated.

OBJECTIVE

- To evaluate the time course and peak response of inflammatory markers, hepatic (Zip14) and duodenal (Zip4) zinc transporters, and the metal binding protein metallothionein-1 (MT-1) following LPS challenge of nursery pigs fed high levels of dietary ZnO and CuSO₄

MATERIALS & METHODS

Experimental Unit: Pig (n = 30)

Blocking: RCBD with 6 pigs/block

Treatment: i.m. LPS (O55:B5) at 12 µg/kg BW on d 23

Time Points: h 0 (baseline), 3, 6, 12, and 24 following LPS

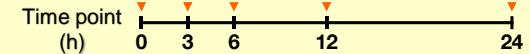
Dietary Mineral Levels: Phase 1 from d 0-7 added 2,500 mg/kg ZnO, phase 2 from d 7-14 added 1,750 mg/kg ZnO, and phase 3 from d 14-23 switched to 200 mg/kg CuSO₄

Data Analysis: Mixed Models for RCBD (1-3), One-way ANOVA (4-5), and orthogonal polynomial contrasts using PROC GLIMMIX of SAS 9.4



Measurement at each time point:

1. Body weight (BW)
2. Rectal temperature (RT)
3. Blood collection for
 - Serum tumor necrosis factor-alpha (TNF-α)
 - Serum C-reactive protein (CRP)
 - Serum zinc and copper concentrations (n = 30, 24, 18, 12, and 6 for h 0, 3, 6, 12, and 24, respectively)



One block of pigs was randomly killed at each time point to collect tissues for quantification of mRNA expressions using RT-qPCR

4. Hepatic IL-6, Zip4, and MT-1
5. Duodenal IL-6, Zip14, and MT-1 (n = 6 at each time point)

RESULTS

Pigs exhibited signs of anorexia, vomiting, and hyperventilation following LPS challenge

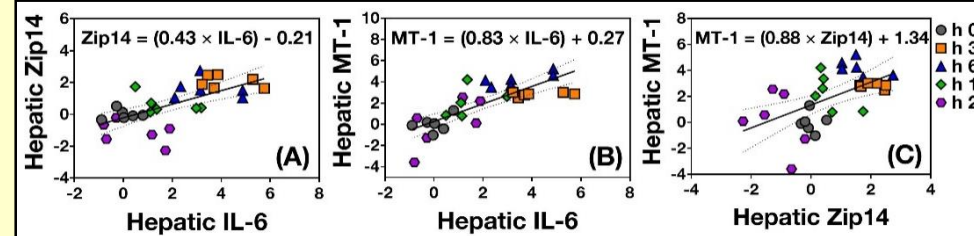


Figure 2. Correlation and linear regression in pairwise comparisons of hepatic genes for the five-time points following LPS challenge.

(A) Positive correlation of Zip14 to IL-6 (r = 0.65, R² = 0.40, P = 0.0001).

(B) Positive correlation of MT-1 to IL-6 (r = 0.78, R² = 0.59, P < 0.0001).

(C) Positive correlation of MT-1 to Zip14 (r = 0.55, R² = 0.28, P = 0.0017).

Figure 1. Time course and peak response of inflammation and hepatic genes mRNA expression of LPS-challenged pigs.

- (A) No change in BW (P > 0.05), but increase in RT (P < 0.05) indicating fever following LPS up to h 6.
- (B) A marked increase (P < 0.0001) in serum TNF-α up to h 3, and a linear increase in serum CRP (P = 0.10) for 24-h period following LPS.
- (C) Decreases in serum zinc (-80%) and copper (-30%) (P < 0.01) following LPS up to h 6 and h 12, respectively.
- (D) Upregulation (P < 0.01) of hepatic IL-6, Zip14, and MT-1 genes following LPS up to h 6 for IL-6 and Zip14, and up to h 12 for MT-1.

CONCLUSIONS

- Our data indicate peak inflammatory responses from 3 to 6-h following LPS challenge for fever, decrease in serum zinc and copper, increase in serum TNF-α, and upregulation of hepatic IL-6, Zip14, and MT-1 genes
- C-reactive protein (CRP) increases linearly over a 24-h period.
- Hepatic IL-6, Zip14, and MT-1 play a rapid major role in inducing hypozincemia (decrease in serum zinc concentration) with strong positive relationships during inflammation (Figure 3).
- Duodenum, a primary site of zinc absorption, its genes are not responsive to LPS challenge.
- Overall, pigs recover to normal stage within 24-h following the challenge.

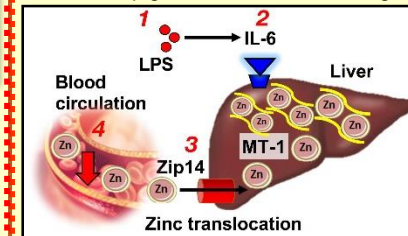


Figure 3. Hypozincemia is induced by LPS upregulating hepatic IL-6, Zip14, and MT-1 mRNA expression during inflammation to increase hepatic zinc uptake and sequestration (adapted from Liuzzi et al., 2005).