

# ***Pig oxidative stress model: Behavior as a potential indicator of oxidative stress in pigs***

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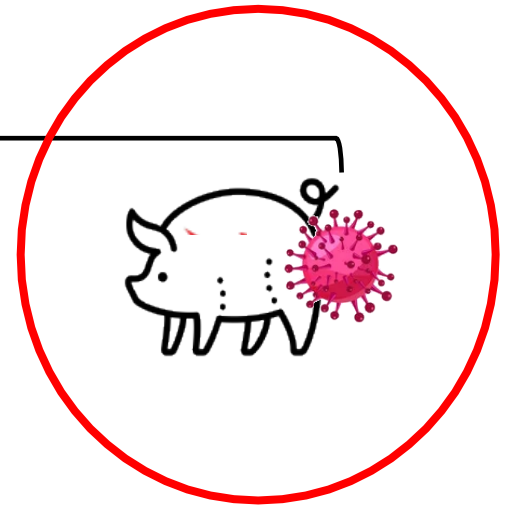
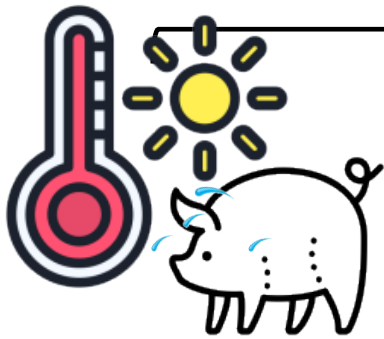
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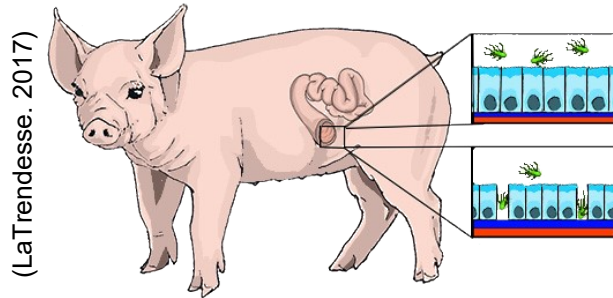


# Pig stress



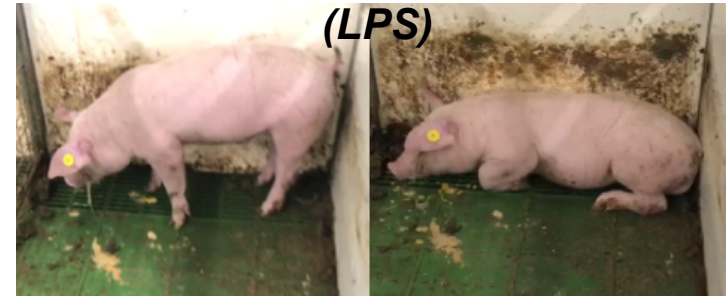
# Oxidative stress

## Oxidative unbalances



- **Free radicals** > Antioxidant capacity
- Intestinal permeability
  - ↓ Nutrient absorption ↑ Infections

## Lipopolysaccharide (LPS)



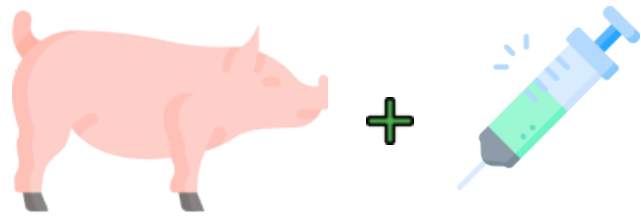
- Potent activator of the immune system
- Current pig study models are **not standardized**
  - Dose (60 – 100 µg/Kg BW)
  - Challenge time (age)

# *Objective*

- To refine an **oxidative stress** model with an **LPS dose** sufficient to trigger a measurable **physiological response** while minimizing animal discomfort (**minimum dose**) on four independent trials.



# M&M: Oxidative challenge



4 hours post injection







Each hour post injection



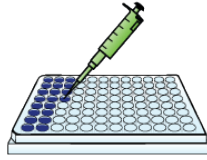
- Intraperitoneal injection
  - Control (**CON**): Saline solution
  - Lipopolysaccharide (**LPS**)

*(Hou et al. 2014; Sakumoto et al. 2003)*

# ***M&M: Experimental design***

	<b>Trial A</b>	<b>Trial B</b>	<b>Trial C</b>	<b>Trial D</b>
	24	24	24	20
 (d.p.w.)	21	21	28	41
 ( $\mu\text{g}/\text{KgBW}$ )	50	50	25	25
	6 pigs/pen	6 pigs/pen	6 pigs/pen	Individual

# M&M: Analyses



- Gene expression was measured through real-time PCR method.
- Gpx, SOD, CAT, and MPO activities were measured through ELISA commercial kits.



- Gene exp., antioxidant act., and MPO
  - One-way ANOVA (Proc GLM):  
Treatment (Ctrl & LPS)
- Behavior observations
  - Logistic model (Proc Logistic):  
Treatment (Ctrl & LPS)  
Time (60, 120, 180, 240)
- $\alpha = 0.05$ ; (Mean  $\pm$  SEM)

# Plasmatic AOX activity: CON vs. LPS



	Trial A (50µg/KgBW; 21 d)	Trial B (50µg/KgBW; 21 d)	Trial C (25µg/KgBW; 28 d)	Trial D (25µg/KgBW; 41 d)
GPx				
SOD				
GST				
CAT				

- $P < 0.05$
- $0.1 \geq P \geq 0.05$

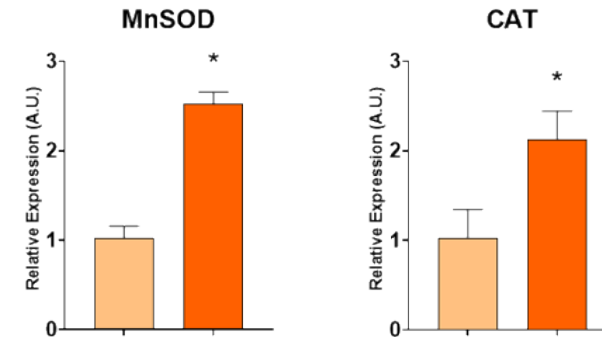
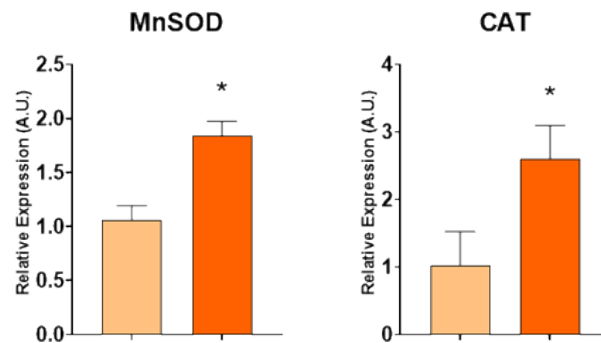
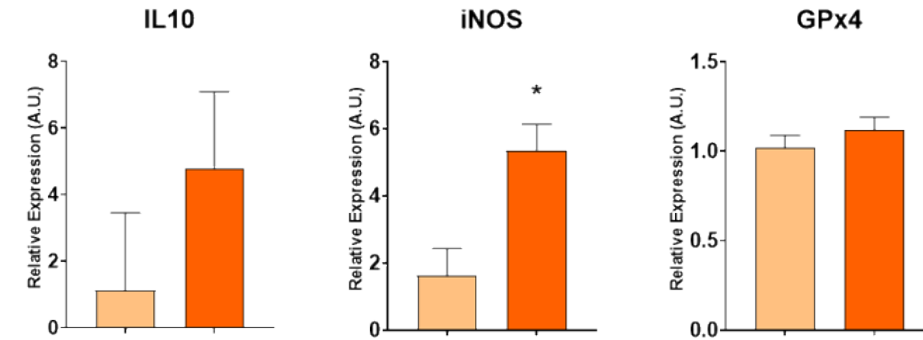
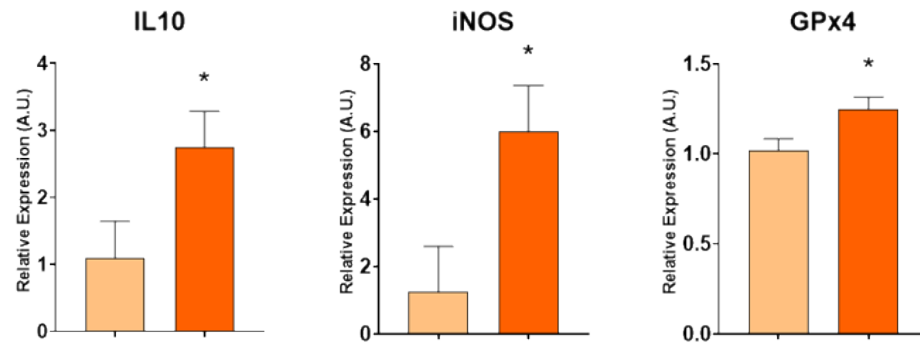
- Plasmatic antioxidant markers response did not show a clear pattern across the trials.



# Gene expression:

Trial A: 50µg/KgBW; 21 d

Trial B: 50µg/KgBW; 21 d



CON LPS

# Fecal myeloperoxidase (MPO):

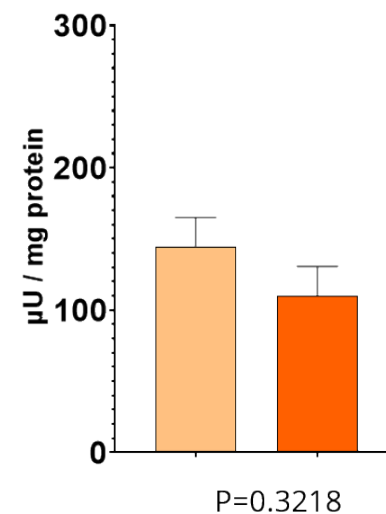
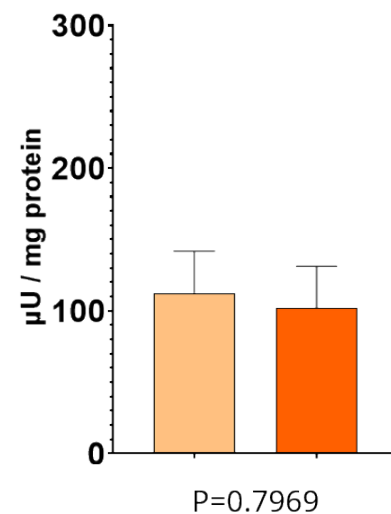
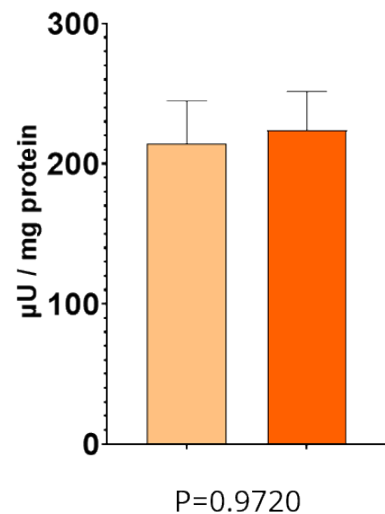


Stress → inflammation of intestines → **MPO activity** ↑ (Rodrigues et al. 2021, Pulli et al., 2013).

Trial A: 50µg/KgBW; 21 d

Trial C: 25µg/KgBW; 28 d

Trial D: 25µg/KgBW; 41 d



CON LPS

- The physiological stress response might have been missed at sampling (Reimert et al. 2014; Němečková et al. 2022).
- Oxidative challenges with mild LPS doses might not trigger intestinal inflammation.
- Feces might not be the proper measurement matrix.



# ***Behavioral observations:***

- Behavior is highly sensible to oxidative challenges (*Tuchscherer et al. 2006*).
- Potential for the development of remote monitoring systems.

■ CON ■ LPS

# ***Conclusions:***

- Animal research models must guarantee the best welfare conditions possible. Refinement of the study models is needed (e.g. Less-invasive monitoring methodologies).
- Oxidative response was associated with the magnitude (LPS dose) and the matureness of the pigs' immune system (age). However, the physiological response was not consistent.
- Behavior may be used as a marker for continuous and non-invasive monitoring of piglets, which is sensible enough for early detection of immune-related problems.



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