Elaboration of an experimental model of the oxidative stress in weaned piglets

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Study context

Pig weaning

- Downgraded sanitary status
- Stress – immune stimulation - infections
- Inflammation

- Oxidative stress
- ROS \( (H_2O_2, O_2^-, OH^-) \)

- Amino acid requirements
- Inflammatory proteins (haptoglobin...)

- Catabolism: FCR
- Tryptophan
- Feed intake

- Robert et al., 2009; Degroote et al, 2012; Michiels et al, 2013
- Pié et al, 2004; Zhu et al, 2012
Study context

- **Oxidative stress in weaned piglets**
  - Imbalance between oxidants (reactive oxygen species) and mechanisms of defense (antioxidant network), involved in pathological conditions (Lykkesfeldt & Svendsen, 2007)

- **Blood parameters proposed for monitoring of porcine oxidative stress** (Guillou et al., 2009; Robert et al., 2009; Michiels et al., 2013):
  - Blood resistance to controlled free radical attack, concentrations in terminal oxidation products, acute phase proteins or antioxidant enzymes.

- **but large individual variations** (Marco-Ramell et al., 2011; Michiels et al., 2013) ➔ difficult to standardize *in vivo* experiments
Study context: stress factors

- **Heat stress vs thermoneutrality**
  - heat: → respiratory frequency and heart rate
  - respiratory mitochondrial chain: electron leakage and superoxide anion production

  ![Mitochondrial Chain Diagram](image)

  [From Aurousseau, 2002]

- **Vaccination → "oxidative burst": defence against bacterial/viral attacks**

  ![Oxidative Burst Diagram](image)

  Production: $O_2^- \rightarrow OH^- \rightarrow ONOO^-$
  Destruction: $O_2 + e^- \xrightarrow{NADPH oxidase} O_2^-$

  Polysaccharidic capsule of bacteria

  Neutrophils and macrophages

  In the phagolysosome membrane:

Aims of the study

- Develop a reproducible model of oxidative stress in weaned piglets using two stress factors:
  - vaccination PCV2 /+ swine influenza at weaning (d1)
  - heat stress

- Effects of an antioxidant combination in phase 1 diet (d1-14)

⇒ Determine the most accurate blood biomarkers of oxidative stress
Materials et methods: stress factors & antioxidant supplementation

- **Challenges vs control**
  - vaccination at weaning (d1)
    - Porcine Circovirus type 2 (PCV2)  
    - PCV2 + swine influenza  
    - heat stress
      - 36.5°C for 6 h at d 9-10, d 23-24, d 36-37
  - **Trial 1**
  - **Trial 2**

- **dietary antioxidant concentration**  
  - **Trial 1 & Trial 2**
    - Phase 1 & 2 (d1-41) standard antioxidant levels (NRC 2012)
      - 0.1 mg/kg Se selenite
      - 16 IU vitamin E
    - Phase 1 (d1-14) high antioxidant levels
      - 0.1 mg/kg Se selenite + 0.2 mg/kg Se yeast (Alkosel®)
      - 100 IU vitamin E
      - 30 mg/kg superoxide dismutase (SOD) rich melon supplement (Melofeed®)
Materials et methods: sampling & measurements

Trial 1

- d0-d1
- d13 d14
- d28 d29
- d40 d41

Trial 2

- d0 d1
- d9-d10 d13 d14
- d23-d24 d28
- d36-d37 d40 d41

sevrage

- High Phase 1
- Low Phase 1/2

- Blood sampling
- Weighing
- Vaccination
- Heat stress (36.5°C sur 2×6h)

Performances & blood parameters

- Performances: weighing at d0, 14, 28/29 et 41, DFI, ADG
- Blood samplings: d 13, (28), 40 × 3 tubes:
  - Serum separator gel → haptoglobin concentration
  - Lithium heparin → glutathione peroxidase activity (GPx)
  - EDTA K2E → oxidation products and antioxidant activity

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Biomarkers tested in study

- **Acute Phase Protein**: haptoglobin [see also Pig-MAP, CRP, cytokines]
  - marker of general health status (Le Floc’h et al. 2004)
  - able to bind haemoglobin, free Hb → oxidative activity (Humblet & Godeau, 2005)
  - Hp response more persistent in time ↔ our protocol with distant blood tests.

- **Oxidation products of lipids, proteins, [DNA]** (Lykkesfeldt & Svendsen, 2007)
  - lipid peroxidation of PUFA (insertion of O₂ group)
  - damages on cell membranes → permeability, fluidity, enzymes or receptors activity
  - protein carbonyls ↔ result of non-specific free radical-mediated oxidation (C=O group) of amino acids
    - functionality of modified protein [3-dimensional structure].
    - carbonyls are stable → analysis.
Biomarkers tested in study

- **Enzymes involved in antioxidant defences**
  - GPx [see also SOD, catalase, GRx].
  - Cofactors: Se → GPx, Zn, Cu or Mn → SOD (Robert et al., 2009).

- **Total antiradical potential**
  - Free radical generator operating under controlled conditions (Prost, 1989)
  - Extracellular and intracellular antioxidant defenses contribute to maintain blood cell membrane integrity and function until cell lysis.
    - The measurement of half-haemolysis time: HT50 is reproducible and representative of overall defense against free radicals in humans and animal models (Guillou et al., 2009; Rossi et al., 2013)
Results: Performances

 Trial 1

- Culled or retired: 3.6%
- Poor effects of treatments
  - High AOX diet ➔ phase 1 DFI
  - PCV2 vaccination ➔ FCR d 0-41

 Trial 2

- PCV2 + influenza vaccination
  - (勋) DFI phase 2 period
  - ➔ ADG phase 2 period for piglets fed low AOX diet (tendency for interaction feed × vaccination)

- Heat stress
  - ➔ d 28-41 DFI
  - ➔ phase 2 period ADG
Vaccination effects

Trial 1
PCV2

Trial 2
PCV2+ influenza

haptoglobin

GPx activity

**VxD**

no vaccination

vaccination

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Vaccination effects

Trial 1
PCV2

Trial 2
PCV2+ influenza
Vaccination effects

Trial 1
PCV2

Trial 2
PCV2+ influenza

H50 of Whole Blood and Red Blood Cells

= blood resistance to free radicals
Trial 2

Heat stress effects

### Haptoglobin
- **g/l**
- **Control**
- **Heat stress**

### GPx activity
- **UI/l**
- **d13**
- **d40**

### Lipid peroxides
- **μmol/l**
- **d13**
- **d40**

### Carbonyl proteins
- **μmol/g protein**
- **d13**
- **d40**

*ST × D **

- **t**
- ****
Trial 2  

Heat stress effects

Lipid peroxides

μmol/l

Heat Stress × Vaccination

HS × V *

control

heat stress

control

heat stress

vaccination

d13

d40

a

b

ab

ab

b
Trial 2

heat stress effects

**H50 of Whole Blood and Red Blood Cells**

- WB
- RBC

**Heat Stress × Vaccination at d40**

**control**
- WB
- RBC

**heat stress**
- WB
- RBC

**vaccination**
- WB
- RBC
Antioxidant effects

Trial 1

Trial 2

= haptoglobin

⇒ GPx activity
Antioxidant effects

- lipid peroxides
- protein carbonyls

Trial 1

Trial 2
Antioxidant effects

Trial 1

Trial 2

 capacità anti-radicalaire
Discussion: influence of stress factors

Effects of vaccination

- Double vaccination (trial 2) vs. single vaccination (trial 1) \( \rightarrow \) responses
  - vaccination challenge vs. disease event
- influenza virus & PCV2 \( \rightarrow \) oxidative stress (Schwarz, 1996; Chen et al., 2012)
- vaccination interacts with other stress factors
  - \( \times \) anti-radical capacity: \( \rightarrow \) favourable effect of antioxidants (trial 1), \( \rightarrow \) HS effect (trial 2)
  - \( \downarrow \) GPx activity & \( \rightarrow \) lipid peroxidation (trial 2)
    - PCV2 \( \downarrow \) GPx activity, also \( \rightarrow \) mRNA levels of GPx, \( \rightarrow \) role of GPx in defense mechanisms against PCV2 (Chen et al., 2012)
Discussion: influence of stress factors

- Effects of Heat Stress (Trial 2)
  - Other studies = HS $\Rightarrow$ production of free radicals and $\Rightarrow$ antioxidant capacity
    - MDA in chicken blood (Altan et al., 2003) and porcine muscle (Yang et al., 2014), MDA modified proteins (Rosado Montilla et al., 2014), lipid peroxides (Altan et al., 2003).
  - no inflammatory response to HS
    - in agreement with Rosado-Montilla et al. (2014).
  - no HS effects before final phase 2 period
    - 36.5 °C $\geq$ thermoneutral zone in early post weaning $\rightarrow$ Too low?
  - no impact on GPx
    - $\neq$ SOD and catalase (Yang et al., 2014) in pig muscle, catalase, SOD and GPx (Altan et al. 2003; Rosado Montilla et al., 2014).
    - in these studies, analysis 1 d after HS = ox reactions and antiox enzymes induced.
      - Rosado Montilla et al. (2014) no production of free radicals or antiox enzymes after 3 d of exposure $= \text{interval between exposure periods and sampling in trial 2} \rightarrow \text{limitation of responses}$?
  - interaction between vaccination x heat stress: interesting results ...
    - No main effect of vaccination on blood anti-radical capacity but HS $\Rightarrow$ HT50 for vaccinated pigs
    - ?? at d13: vaccination $\Rightarrow$ lipid peroxide except for piglets exposed to HS, at d 40: additive effects
    - HS $\rightarrow$ plasma insulin & post-absorption energetic variables (Pearce et al, 2015)
Discussion: Effect of antioxidant nutrients

Phase 1 antioxidant supplementation

- **Phase 1 diet antioxidants = SOD + Se + vit E**

  - → GPx, ↓ oxidation products, ↑ antiradical capacity

  - **SOD from melon concentrate**
    - ↓ stress proteins in gastrointestinal tract after weaning (Lallès et al., 2011).

  - **Selenium = constituent of GPx**
    - inhibitory effect of Se-methionine on PCV2 replication could be explained by → GPx activity (Pan et al., 2008).

  - **Vitamin E = antioxidant consumed during oxidative stress**
    - vitamin E → effect on porcine RBC hemolysis (Young et al., 1976).
    - Se and vitamin E → WB HT50 after a 39-day supplementation (Guillou et al., 2009).
Blood antiradical capacity

- free radical haemolysis test $\leftrightarrow$ antioxidants, ↓ onset of oxidation reactions. HT50 = [antioxidants]$_{\text{blood \& RBC}}$
- HT50 at d 13 similar in both tests
  - > d 11 values of Guillou et al. (2009).
- During post weaning, slight $\approx$ for WB and = for RBC
  - = results of Young et al. (1976) & Guillou et al. (2009).

GPx and enzymes

- RBC rich in Cat, SOD and GPx. [enzymes] $\uparrow$ by moderate oxidative stress but $\approx$ high stress.
- GPx activity kinetics trial 1$\uparrow$ $\approx$ (peak at d 28) ≠ trial 2 $\uparrow$ $\uparrow$
  - gradual correction of oxidative stress weaning (Petrovič et al., 2009)
Discussion: biomarkers

- **Lipid peroxides**
  - lipid oxidation markers complex for oxidative stress assessment because of low [lipids] in piglets after weaning (Robert et al., 2009)
  - [lipid peroxides) comparable at d 13 and 40 of both trials but significant effects of diet and vaccination for trial 2.
  - Trial 1: peak at d 28, as GPx → destruction of peroxidized lipids.

- **Protein carbonyls**
  - Both trials: vaccinated pigs with HA diet ➔ protein carbonyls.
  - common marker of oxidative stress but contradictory results (Lykkesfeldt and Svendsen, 2007).
    - Concentration evolution during stress differs between pigs (Marco Ramell et al., 2011).
    - ➔ carbonyl proteins, following vaccination, ➔ involvement of liver in detoxification mechanisms (Tkachenko et al., 2014).
Conclusions

- Stress Factors
  - Mild effects on zootechnical performances
  - Double vaccination (PCV2/swine influenza) at weaning:
    - haptoglobin, ↓ GPx activity, ↑ lipid peroxides, ↓ protein carbonyls
  - Heat stress: ↓ blood resistance to free radicals for vaccinated piglets
    - Improvements: ↑° C d9-10 ? Blood sampling time?
  - Efficient model to study oxidative stress in weaned piglets
  - Antioxidant combination in phase 1 diet
    - Organic Se + vit E + SOD
    - ↑ blood resistance, ↑ GPx activity, ↓ oxidation products of lipids & proteins
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