LALLEMAND ANIMAL NUTRITION

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

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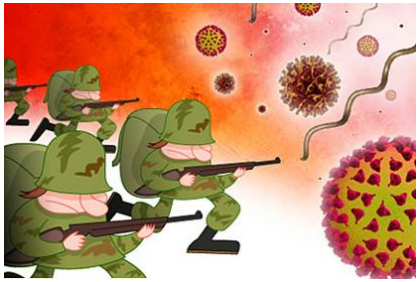
Study context



Pig weaning

downgraded sanitary status ⇄ stress – immune stimulation - infections

stress – immune stimulation - infections → **inflammation**

inflammation → Inflammatory proteins (haptoglobin…)

Inflammatory proteins (haptoglobin…) → Amino acid requirements

Amino acid requirements → **catabolism**: FCR ↗

catabolism: FCR → tryptophan → feed intake ↘

inflammation → **ROS** (H_2O_2, $O_2^{\circ -}$, OH° …)

ROS → **Oxidative stress**

Oxidative stress → downgraded sanitary status

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

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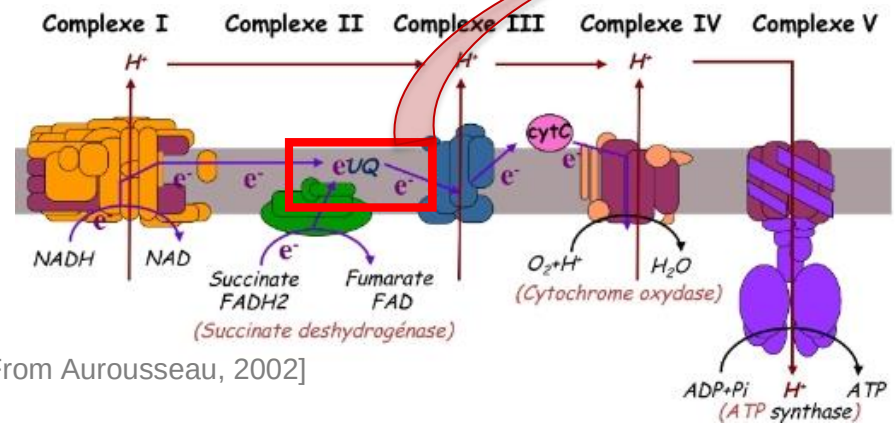
Study context

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

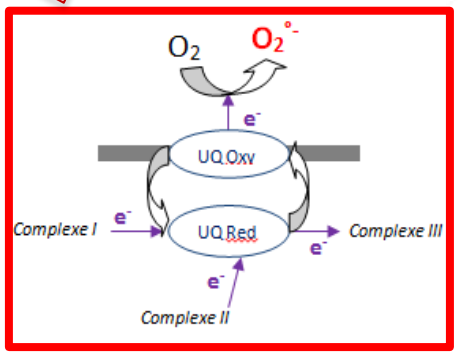
  - Blood parameters proposed for monitoring of porcine oxidative stress (Guillou et al., 2009; Robert et al., 2009; Michiels et al., 2013) :
    - blood resistance to controlled free radical attack, concentrations in terminal oxidation products, acute phase proteins or antioxidant enzymes.
  - but large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



[From Aurousseau, 2002]



- Morrow-Tesch et al, 1994; Hicks et al, 1998; Altan et al, 2000, 2003; Yang et al, 2014; Rosado-Montilla et al, 2014; Pearce et al, 2012, 2013, 2014, 2015

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

neutrophils and macrophages → **Production** → $O_2^{-\circ}$, OH°, $ONOO^-$ → **Destruction** → polysaccharidic capsule of bacteria

In the phagolysosome membrane : $O_2 + e^- \xrightarrow{\text{NADPH oxidase}} O_2^{-\circ}$ (Auberval, 2010)

- Pan et al, 2008; Grau-Roma et al, 2009; Phillips et al, 2010; Chen et al, 2012a-b; Ren et al, 2013; Tkachenko et al, 2014

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

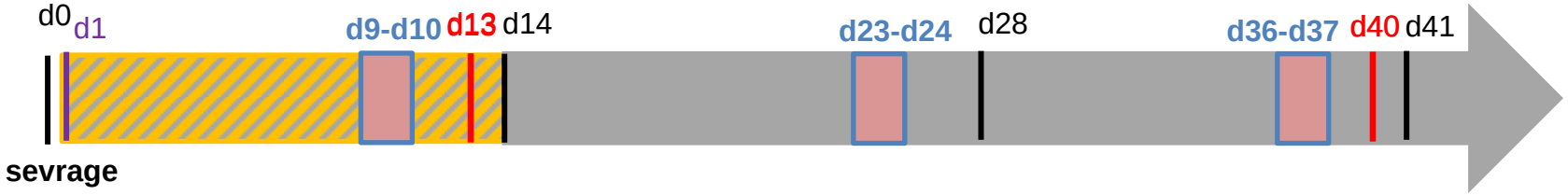
- **Challenges vs control**
  - vaccination at weaning (d1)
    - Porcine Circovirus type 2 (PCV2) — Trial 1
    - PCV2 + swine influenza — Trial 2
  - heat stress
    - 36.5° C for 6 h at d 9-10, d 23-24, d 36-37 — Trial 2

- **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

Trial 2



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

M&M: indicators of oxidative status - 1

- **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 O_2 group)

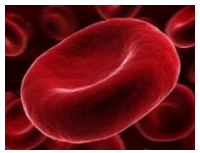
      ⇨ damages on cell membranes ⇨↘ permeability, fluidity, enzymes or receptors activity
    - protein carbonyls ↔ result of non-specific free radical-mediated oxidation (C=O group) of amino acids

      ↘ functionality of modified protein [3-dimensional structure].

      carbonyls are stable → analysis.

M&M: indicators of oxidative status - 2

- **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
    - (↘) 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

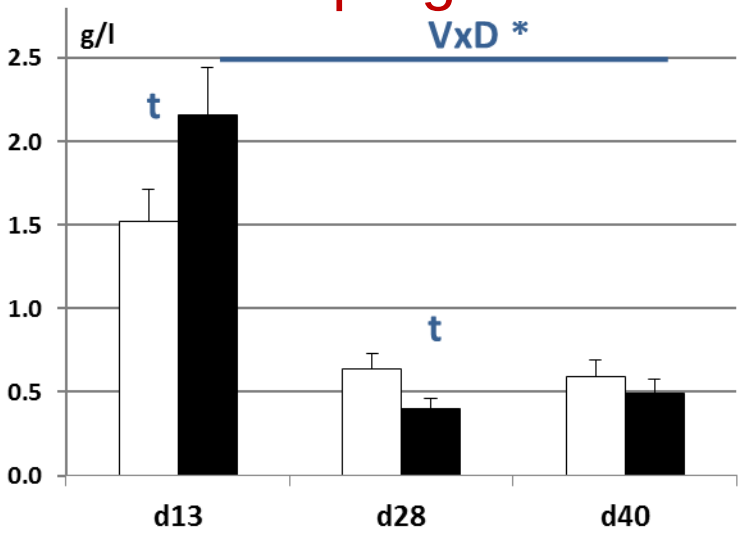
no vaccination

vaccination

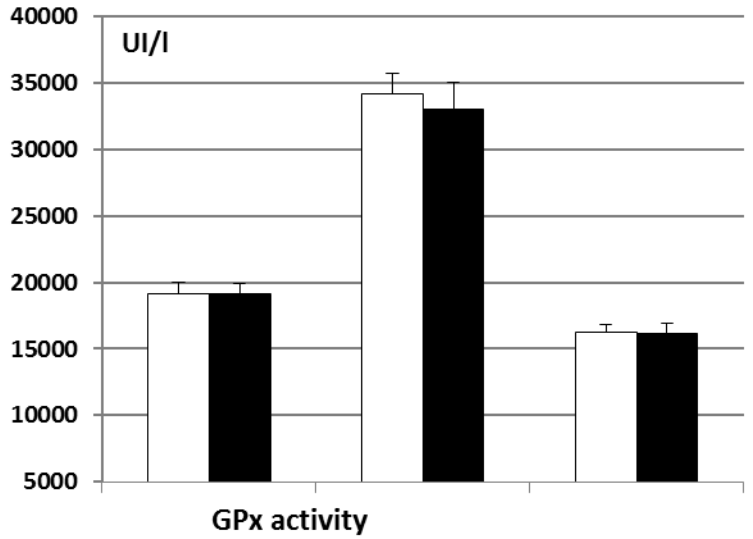
Trial 1

PCV2

↗ haptoglobin

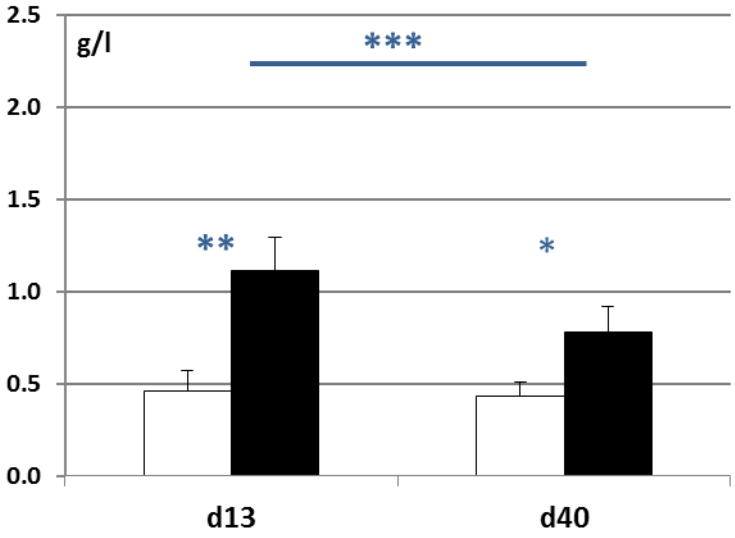


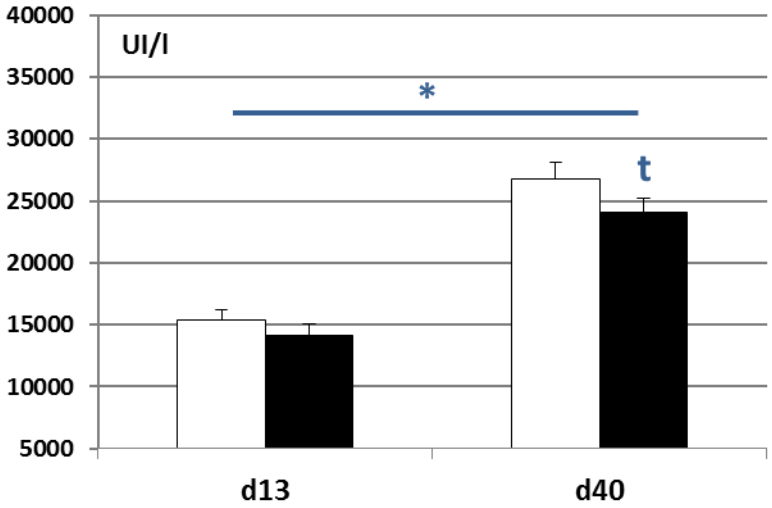
↘ GPx activity



Trial 2

PCV2+ influenza





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Vaccination effects

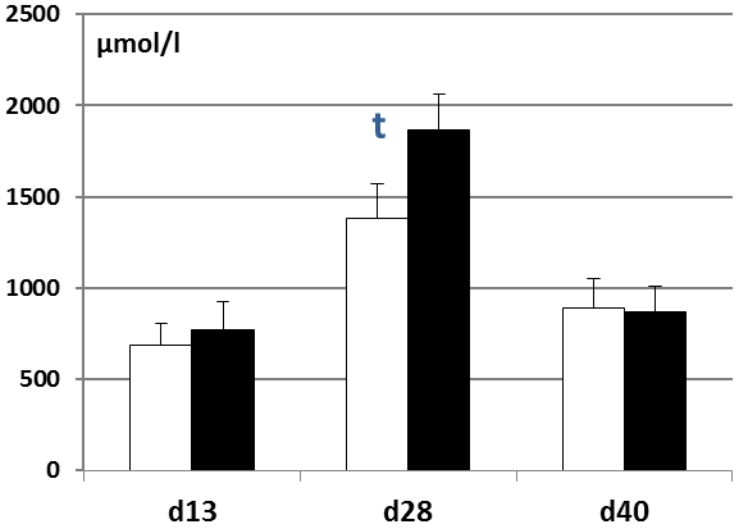
without vaccination

with vaccination

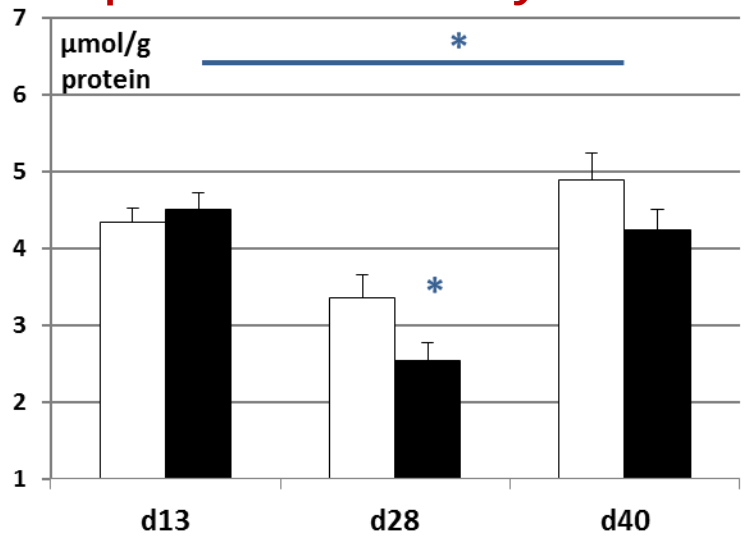
Trial 1

PCV2

↗ lipid peroxides

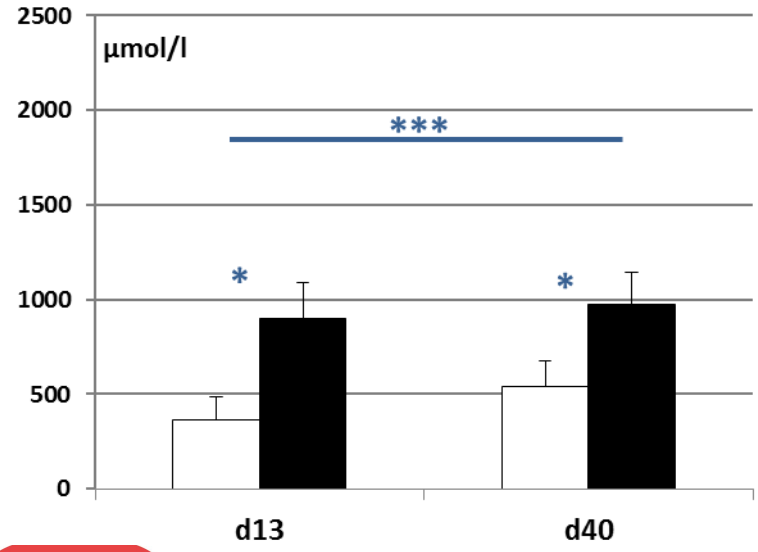


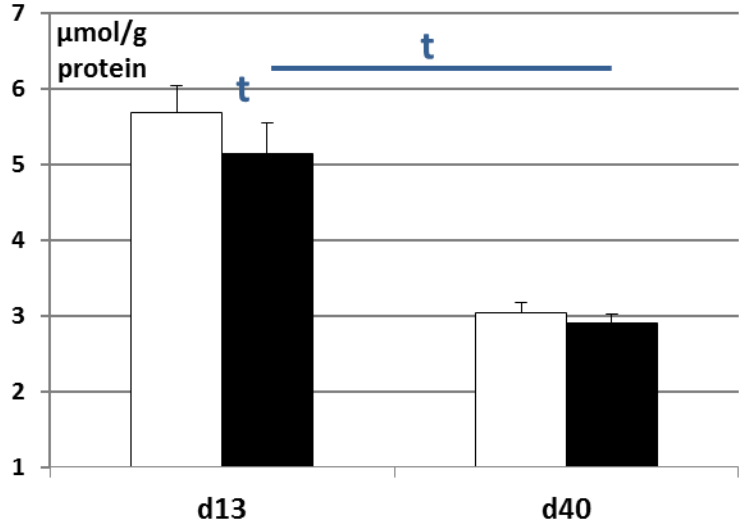
↘ protein carbonyls



Trial 2

PCV2+ influenza





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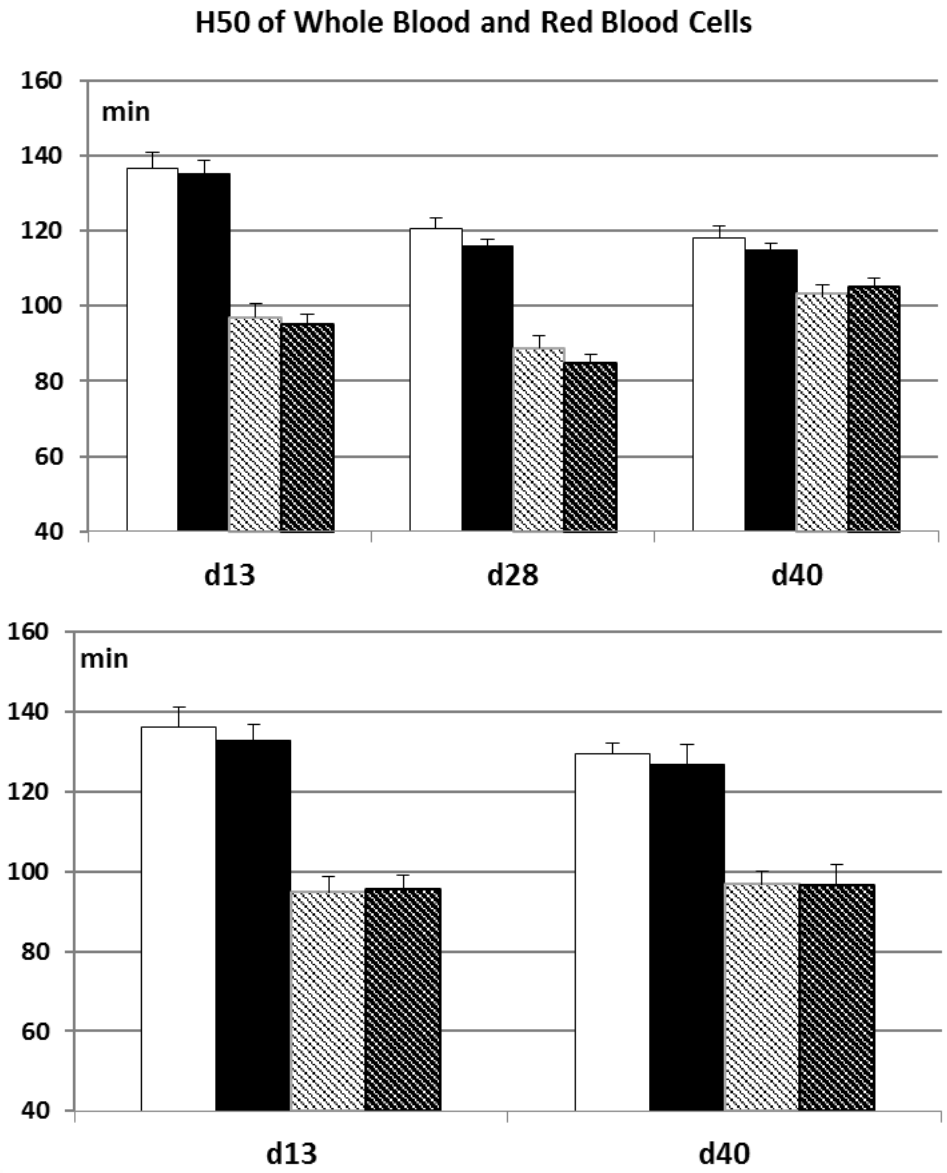
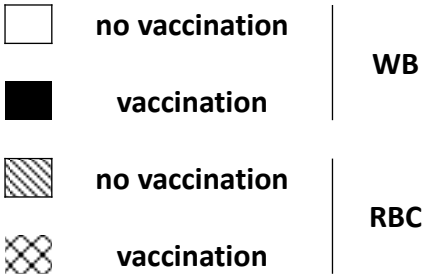
Vaccination effects

Trial 1
PCV2

Trial 2
PCV2+
influenza

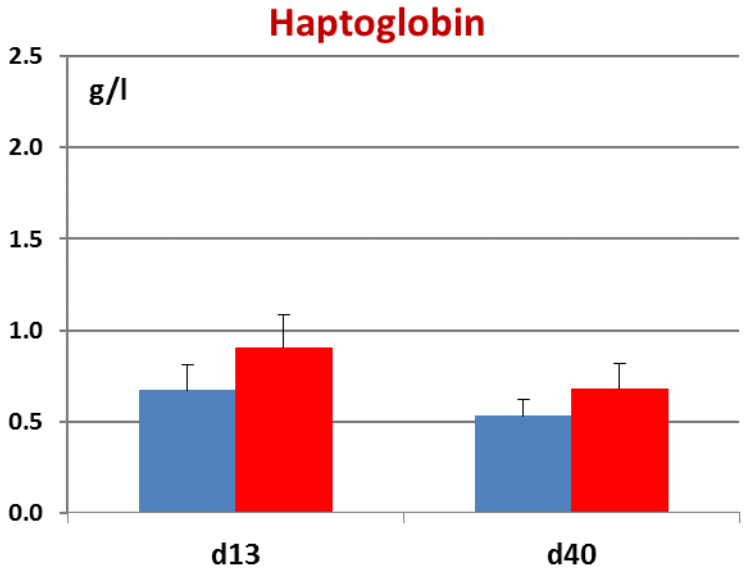


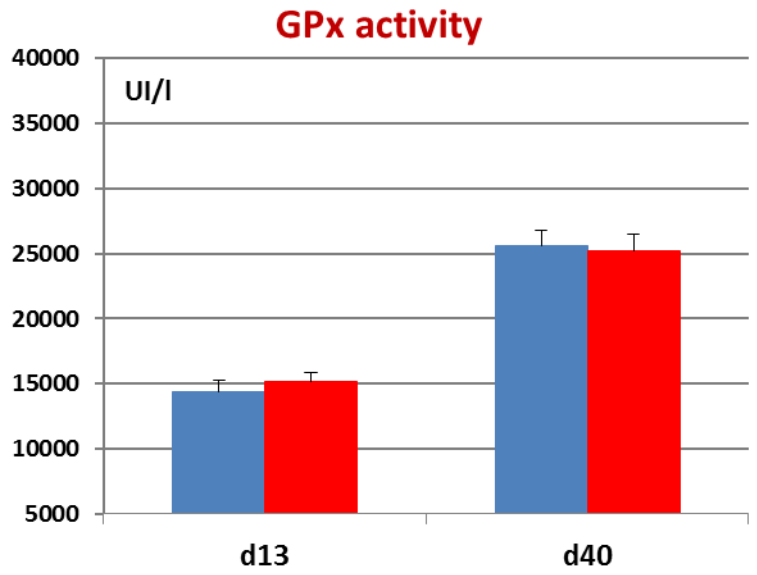
= blood resistance to free radicals



Trial 2

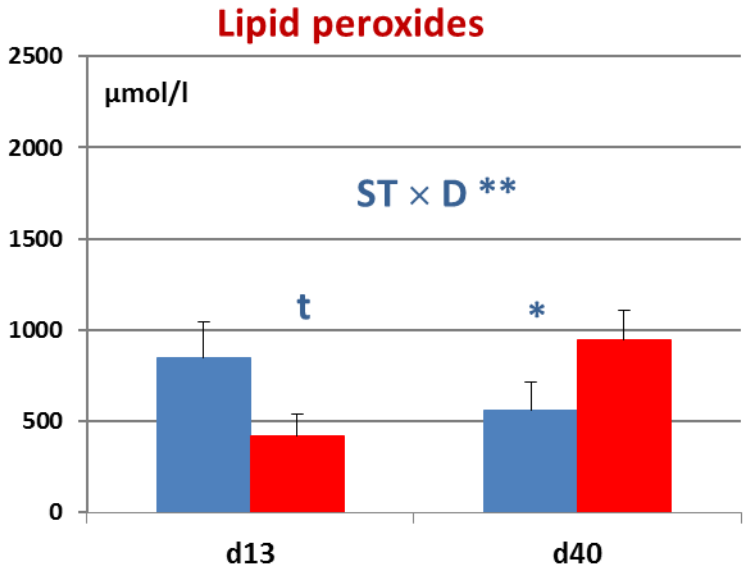
Heat stress effects

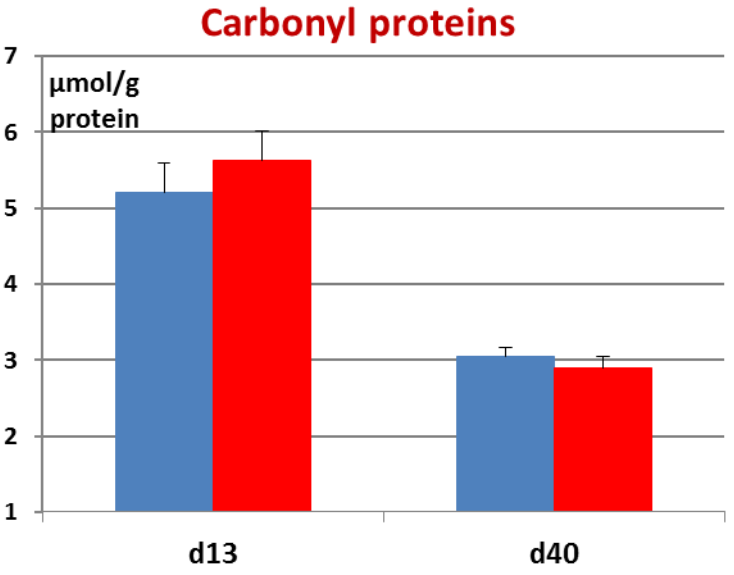




Control

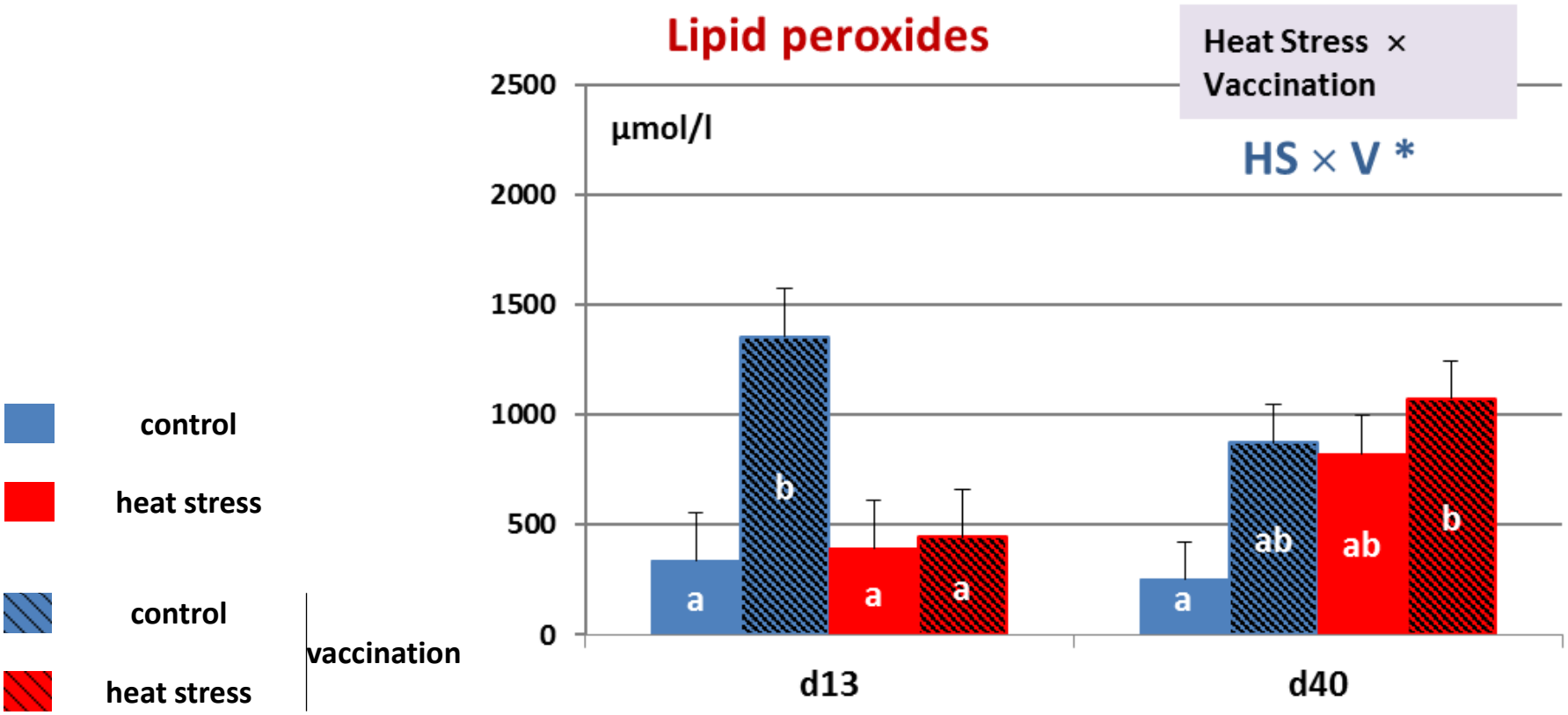
Heat stress





Trial 2

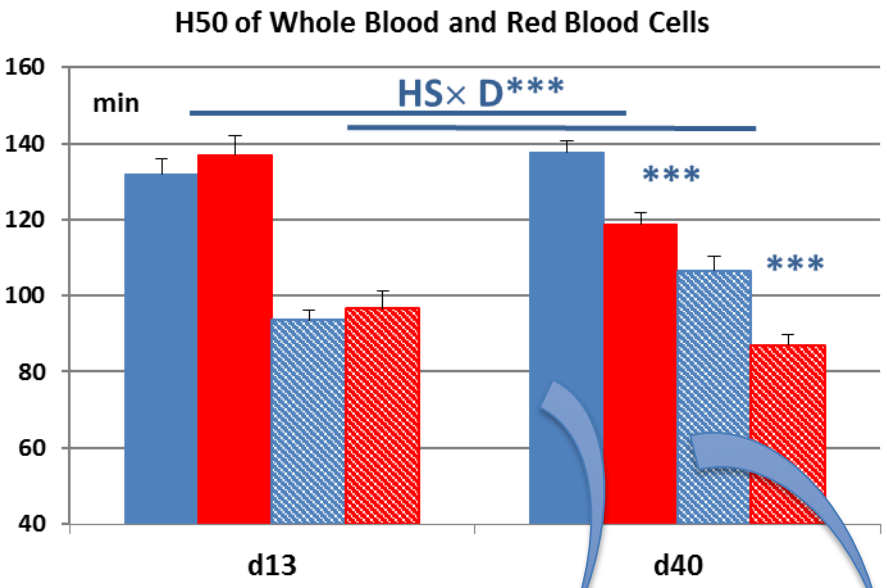
Heat stress effects



Trial 2 heat stress effects

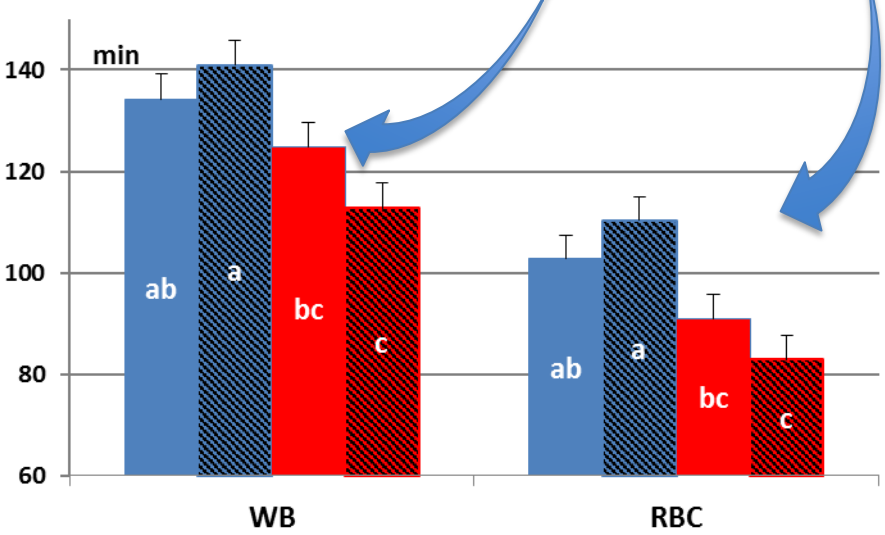
blood resistance

control
heat stress
WB
control
heat stress
RBC



HS × V × D *

control
heat stress
control
heat stress
vaccination



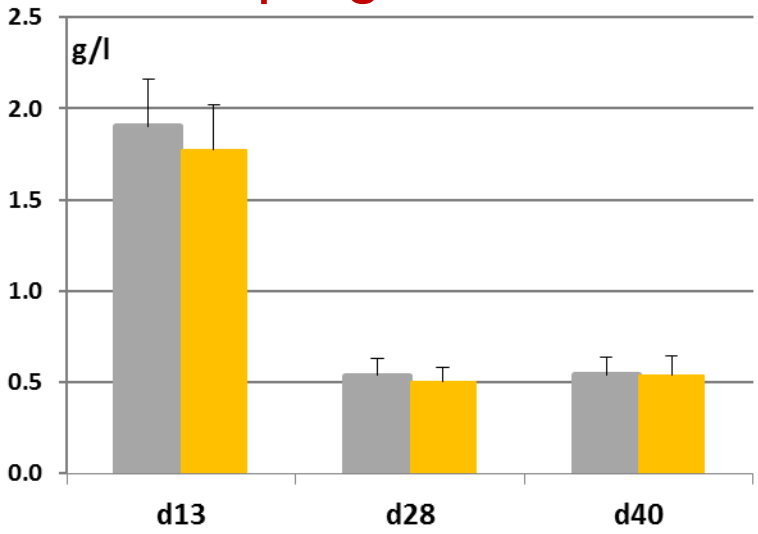
Antioxidant effects

antioxidants -

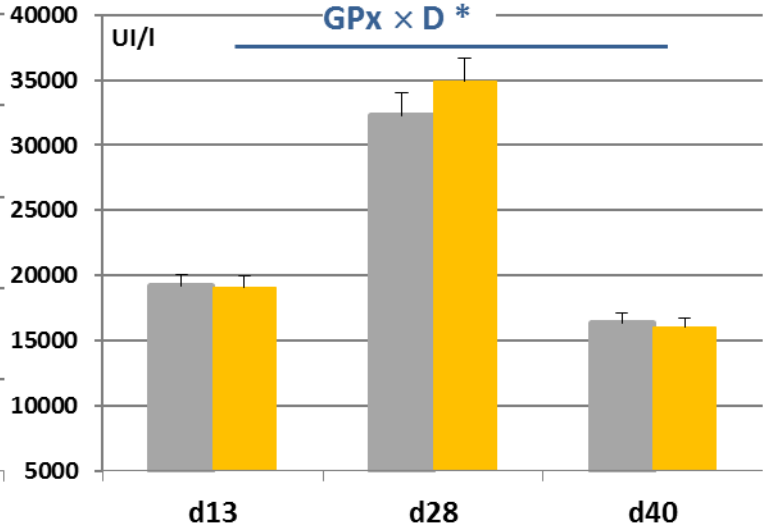
antioxidants +

Trial 1

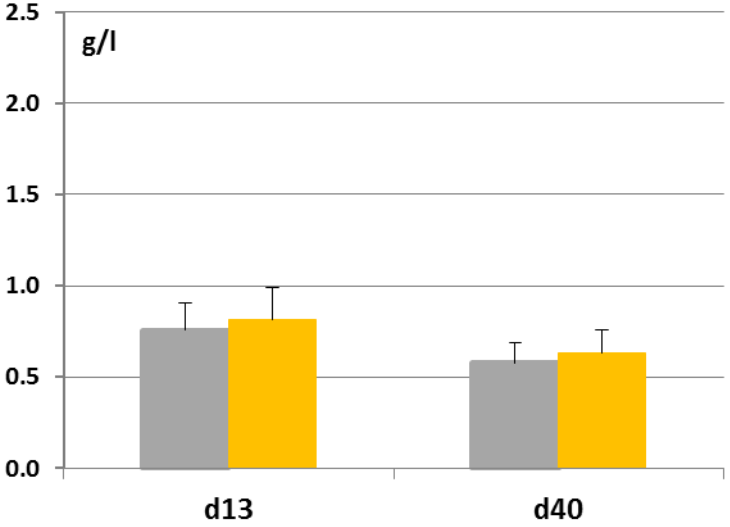
= haptoglobin

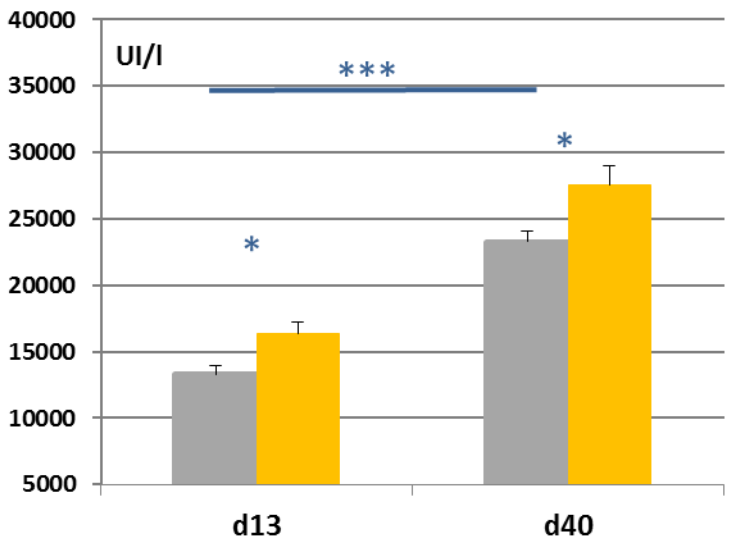


↗ GPx activity



Trial 2





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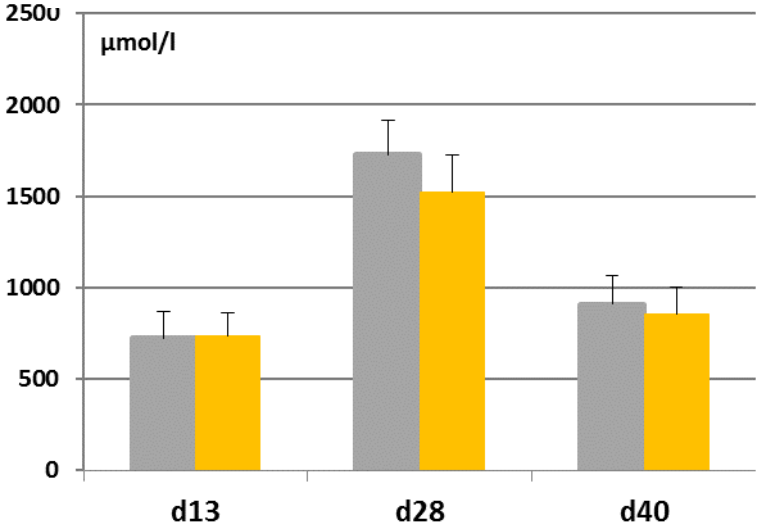
Antioxidant effects

antioxidants -

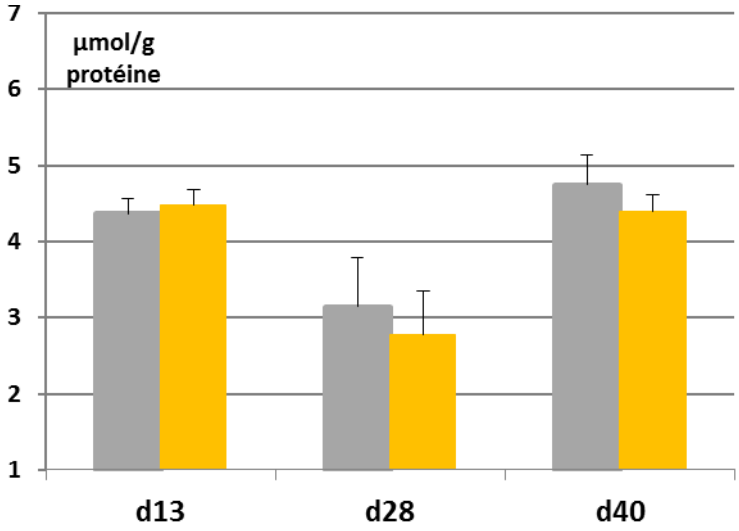
antioxidants +

Trial 1

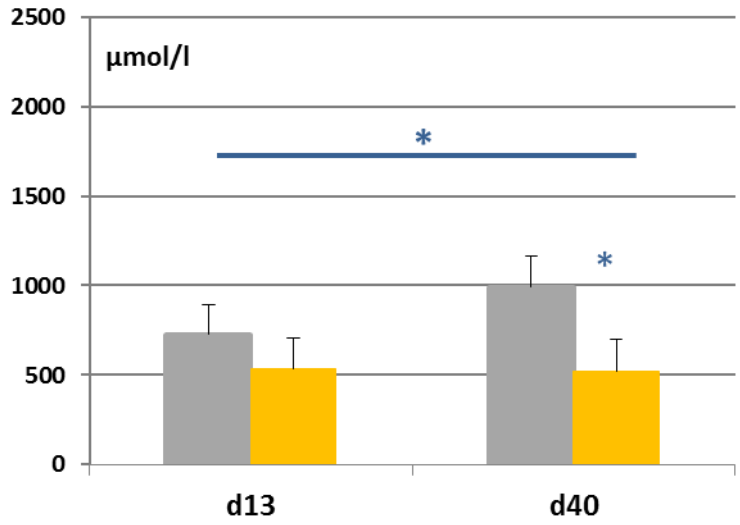
↘ lipid peroxides

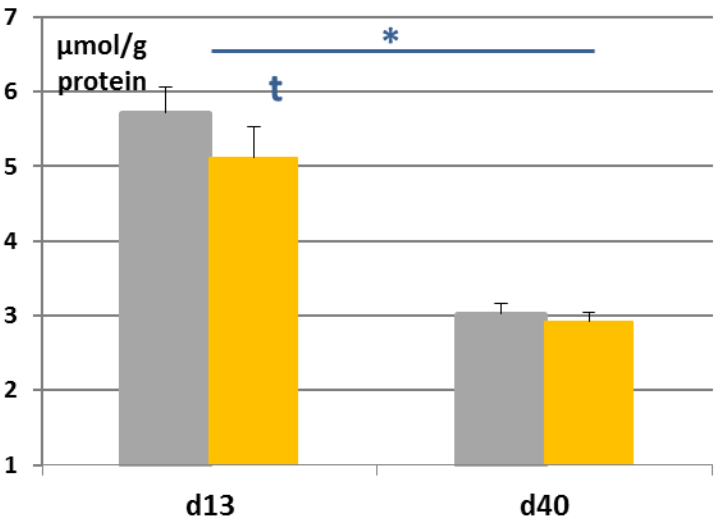


↘ protein carbonyls



Trial 2

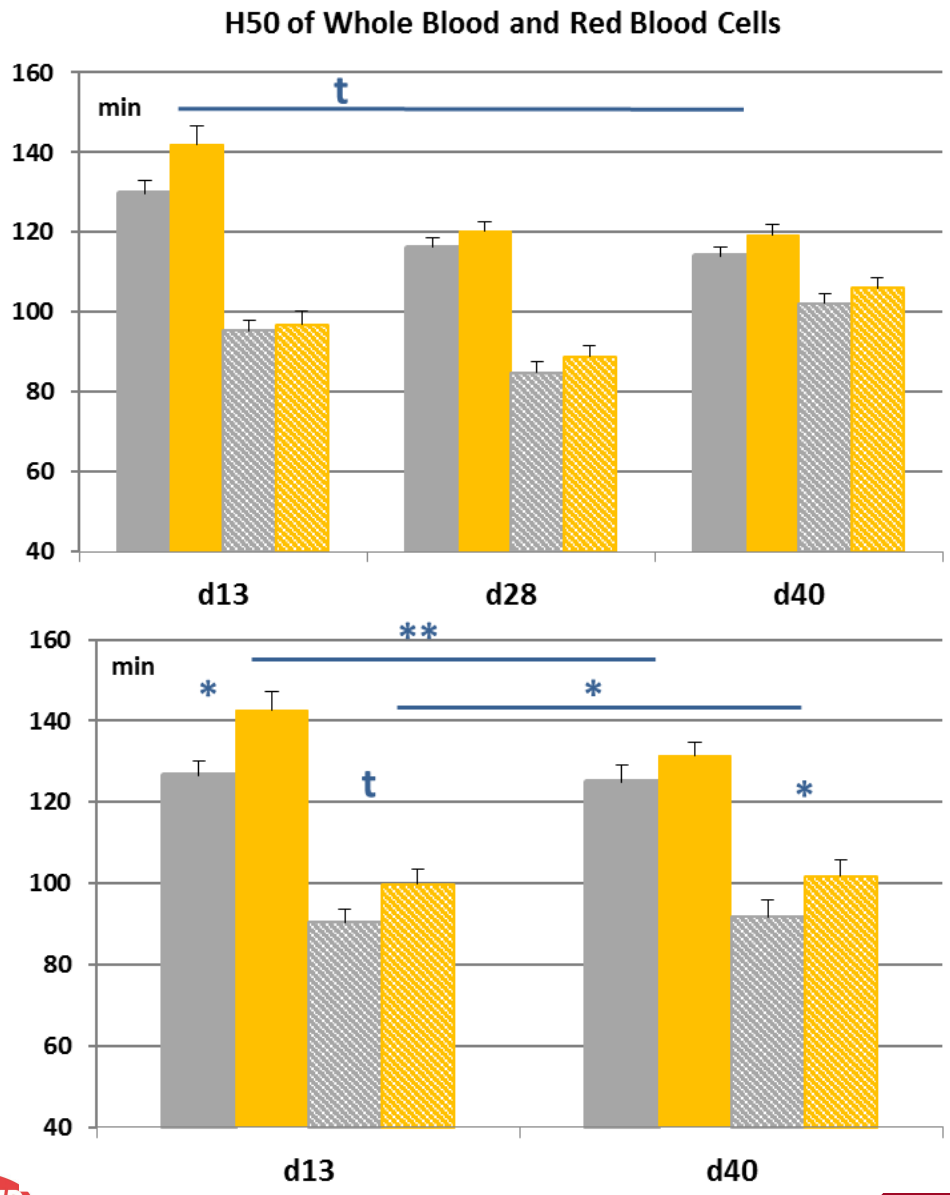




Antioxidant effects

Trial 1

Trial 2



↗ capacité anti-radicalaire

antioxydants - | WB
antioxydants + | WB
antioxydants - | RBC
antioxydants + | RBC

Discussion : influence of stress factors

- Effects of vaccination
  - Double vaccination (trial 2) vs. single vaccination (trial 1) ↗ responses
    - vaccination challenge vs. disease event
  - influenza virus & PCV2 ↗ oxidative stress (Schwarz, 1996; Chen et al, 2012)
  - vaccination interacts with other stress factors
    - × anti-radical capacity: ↘ favourable effect of antioxidants (trial 1), ↗ HS effect (trial 2)
    - ↘ GPx activity & ↗ lipid peroxidation (trial 2)
      - PCV2 ↘ GPx activity, also ↗ mRNA levels of GPx , → 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 ↗ production of free radicals and ↘ 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 ≥ thermoneutral zone in early post weaning → Too low ?
- no impact on GPx
  - ≠ ↘ 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* = interval between exposure periods and sampling in trial 2 → limitation of responses ?
- interaction between vaccination x heat stress : interesting results ...
  - No main effect of vaccination on blood anti-radical capacity but HS ↘ HT50 for vaccinated pigs
  - ?? at d13 : vaccination ↗ lipid peroxide except for piglets exposed to HS, at d 40 : additive effects
  - HS → 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) .

Discussion : biomarkers

- Blood antiradical capacity
  - free radical haemolysis test →|← antioxidants , ⇣ onset of oxidation reactions. HT50 = [antioxidants] blood & RBC
  - HT50 at d 13 similar in both tests
    - > d 11 values of Guillou et al. (2009).
  - During post weaning, slight ↘ 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] ↗ by moderate oxidative stress but ↘ high stress.
  - GPx activity kinetics trial 1↗ ↘ (peak at d 28) ≠ trial 2 ↗ ↗
    - 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

Aknowledgments

- Villefranche-de-Rouergue experimental station
  - A. Vaslin, B. Thomas, G. Roques, K. Marie-Louise-Bredon, S. Pons (GIE Villefranche Grand Sud)
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  - F. Davodeau (Veto d'Oc)

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  - G. Poudevigne (Socsa)

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MINISTÈRE DE L'AGRICULTURE ET DE L'AGROALIMENTAIRE

Thank you for your attention

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