Saliva as a tool to detect chronic stress in piglets

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Applied Veterinary Morphology
University of Antwerp

Introduction: Pigs and stress?

- Why monitoring chronic stress?
  - Animal welfare
  - Economical aspect
    - Zootechnical performances
    - Susceptibility to diseases
    - Impair reproductive capacity
    - …
- How?
  - Body weight, weight gain and feed conversion
  - Behaviour
  - Blood
  - Cortisol in hair
  - Salivary cortisol
  - …

Short communication

Hair or salivary cortisol analysis to identify chronic stress in piglets?

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Abstract

Hair cortisol might better represent chronic stress than salivary cortisol in piglets. To test this hypothesis, 24 female, 7-day old piglets were allocated to two groups and artificially reared. The piglets in the stressed group were exposed to overcrowding (0.10 m^2/piglet) and frequent mixing with unfamiliar piglets until the age of 28 days. The control group remained in an unchanging group at a density of 0.29 m^2/piglet. After 3 weeks, stressed animals had gained significantly less weight (median, here and throughout, 7.58 kg) than the control animals (6.43 kg; $P = 0.021$). Additionally, hair from the stressed group contained significantly higher cortisol concentrations (87.29 vs. 75.60 pg/mg hair; $P = 0.005$), whereas salivary cortisol concentrations did not significantly differ between groups (0.30 vs. 0.25 µg/dL saliva; $P = 0.447$). Weight gain and hair cortisol concentrations were significantly correlated ($P = 0.036$, $r = -0.430$), but neither of these parameters were correlated with salivary cortisol concentrations ($P = 0.929$, $r = 0.019$ and $P = 0.904$, $r = 0.026$, respectively).

Keywords: Chronic stress; Cortisol; Hair; Pig; Saliva

Introduction: Why saliva?

- Is saliva a valuable diagnostic biofluid?
  - Non-invasive technique
  - Stress-free collection
  - No trained staff
  - Rapid results

Protein	Fold change	Type of stressor	Reference
Cortisol	Up	Acute and chronic	Parrot et al., 1989
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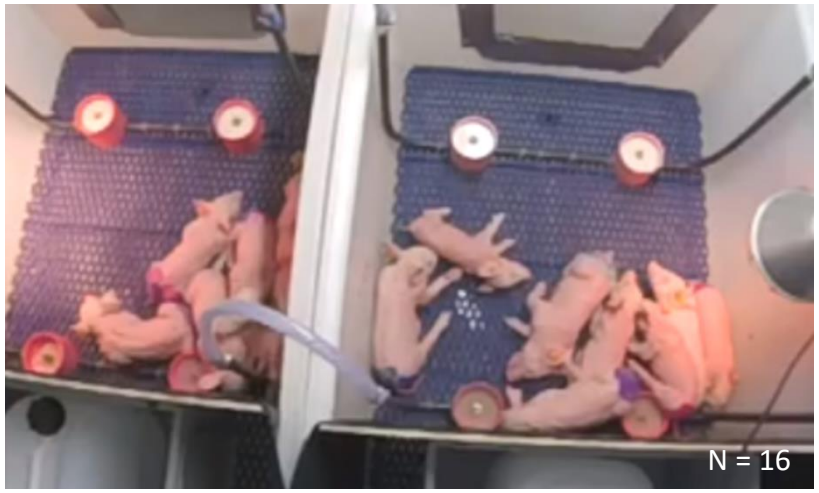
Immunoglobulin M	Down	Acute	Gutiérrez et al., 2017
Double headed protease inhibitor submandibular gland	Up	Acute	Gutiérrez et al., 2017
Haemoglobin	Up	Acute	Gutiérrez et al., 2017

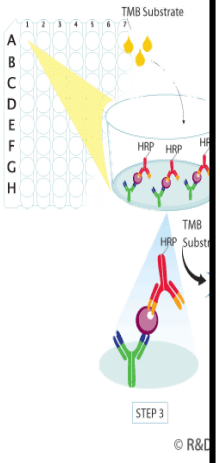
Material and methods: Experimental setup



- Stressors:
  - Overcrowding
    - Control group: 0.29 m^2/animal
    - Stress group: 0.10 m^2/animal
    - Legal minimum: 0.15 m^2/animal (<10 kg)
  - No cage enrichment
  - Mixing with unfamiliar animals
- Parameters:
  - Weight gain
  - Hair cortisol
  - Salivary cortisol
  - Salivary shotgun proteomics

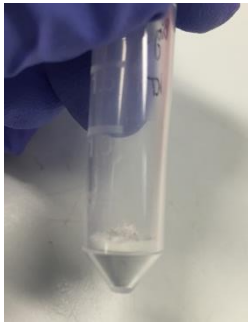
Material and methods: Weight gain and cortisol

7 days of age



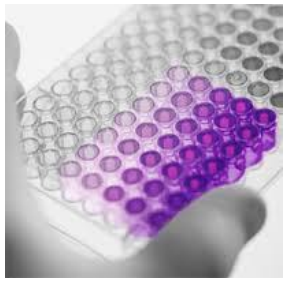
Cortisol E

Cut and grind

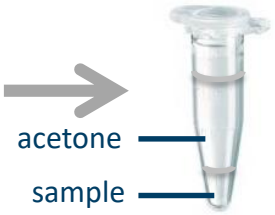


Cortisol extraction

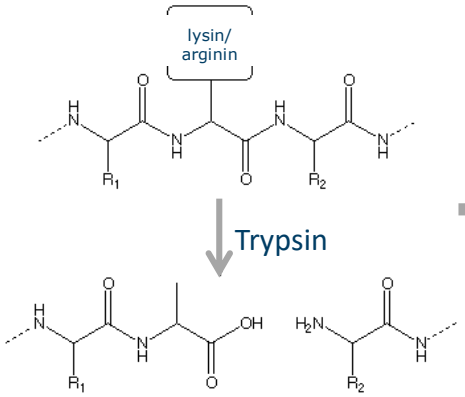
Material and methods: Salivary analysis



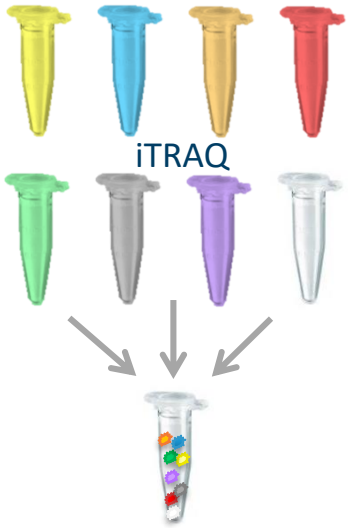
Determination protein concentration



Purification

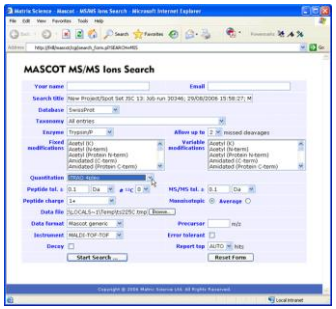


Denaturation and digestion

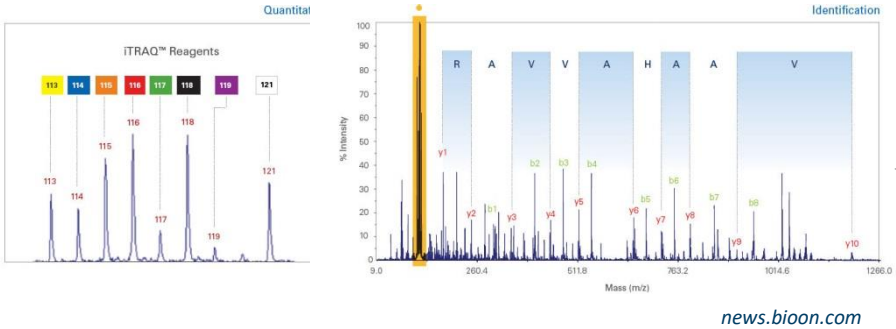


Labelling

2D separation of peptides: SCX-HPLC and RP-HPLC



Identification: MASCOT and Scaffold



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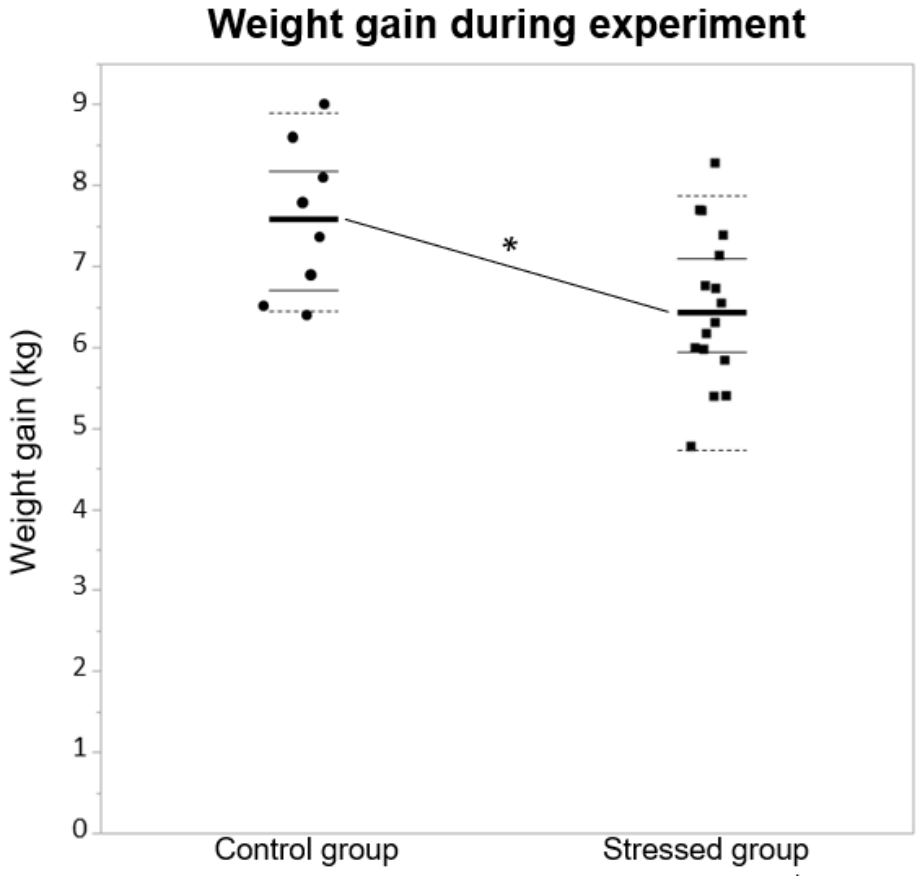
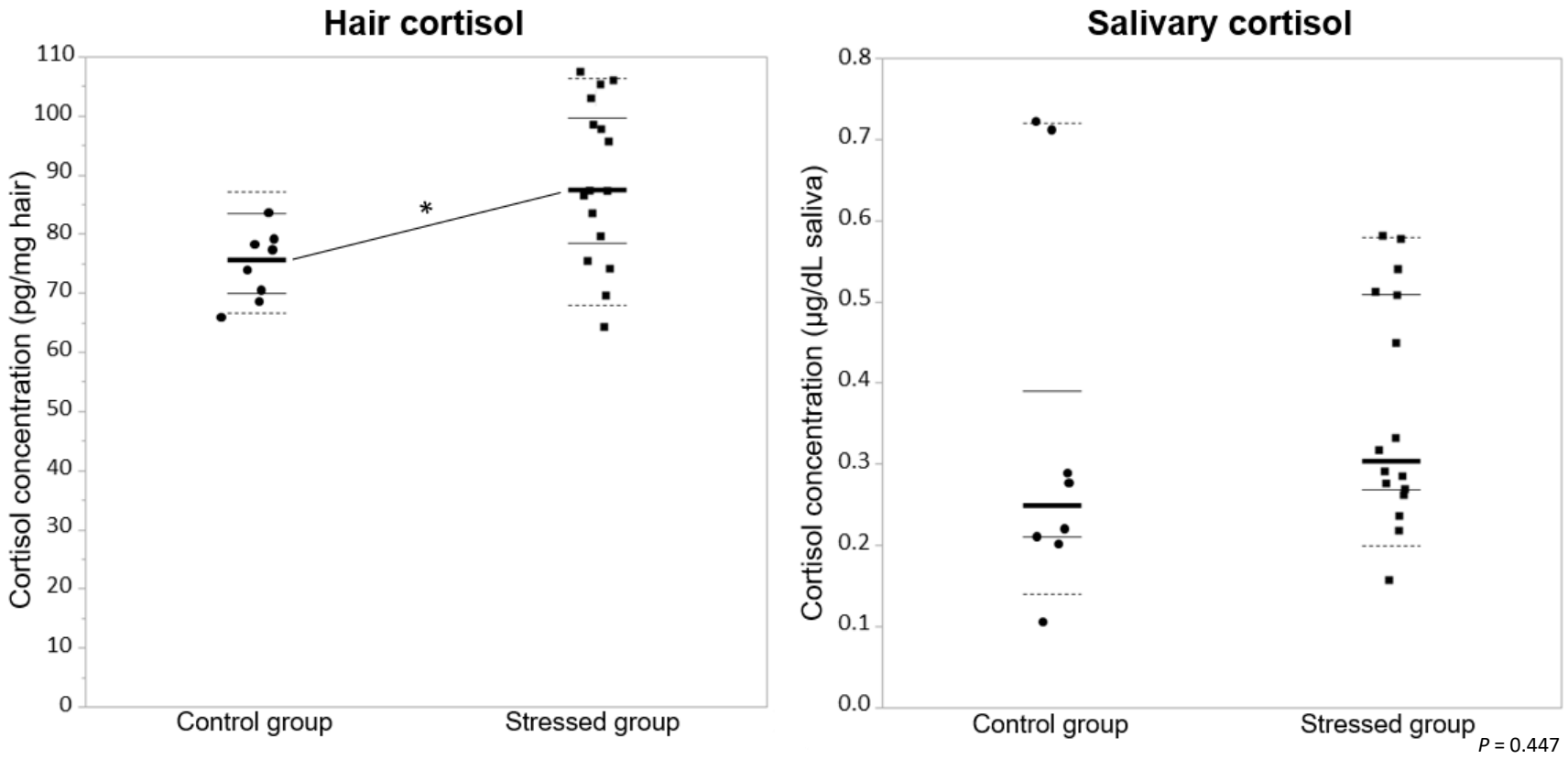
Characterisation and relative quantification: ESI-LTQ-Orbitrap MS/MS

Results: Weight gain



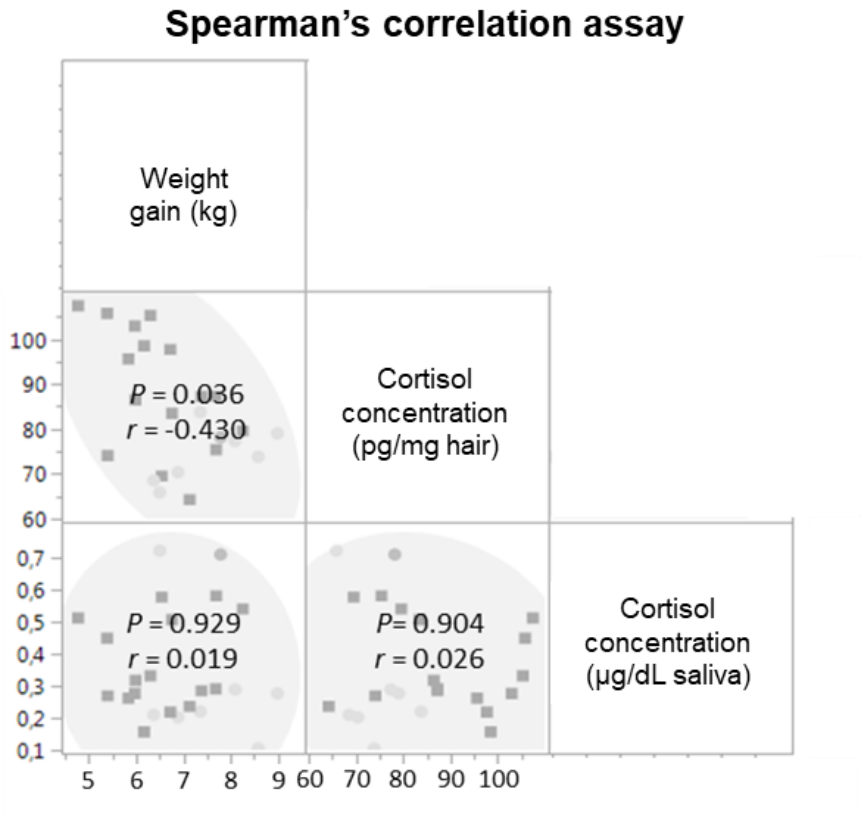
*P = 0.021 (linear mixed models, $P \leq 0.05$). For each group the median (thick line), the 25th and 75th percentile (thin lines) and the 5th and 95th percentile (dotted lines) are shown.

Results: Cortisol concentrations



*P = 0.005 (linear mixed models, $P \leq 0.05$). For each group the median (thick line), the 25th and 75th percentile (thin lines) and the 5th and 95th percentile (dotted lines) are shown.

Results: Correlation assay

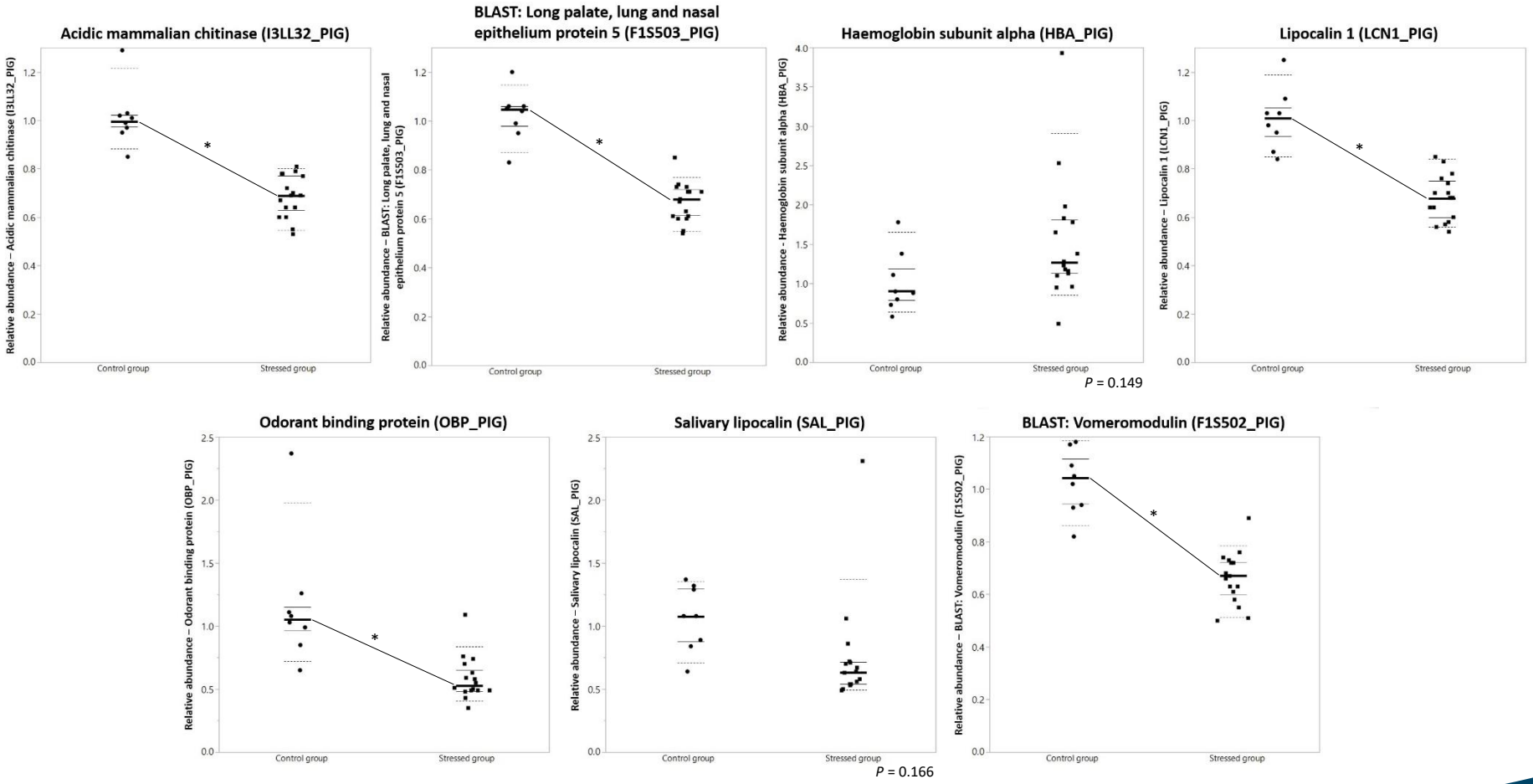


Results: Salivary shotgun proteomics

- 596 proteins identified
- 7 proteins with a significant (>1.4) fold change

	Name protein	Accession number	Number of unique peptides	Fold change	Up or down
1	Acidic mammalian chitinase	I3LL32_PIG	7	1.49	↓
2	BLAST: Long palate, lung and nasal epithelium protein 5	F1S503_PIG	8	1.53	↓
3	Haemoglobin subunit alpha	HBA_PIG	7	1.51	↑
4	Lipocalin 1	LCN1_PIG	10	1.48	↓
5	Odorant binding protein	OBP_PIG	9	1.99	↓
6	Salivary lipocalin	SAL_PIG	11	1.41	↓
7	BLAST: Vomeromodulin	F1S502_PIG	16	1.54	↓

Results: Salivary shotgun proteomics



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BLAST: Vomeromodulin	**Down**	**Chronic**	
BLAST: Long palate, lung and nasal epithelium protein 5	**Down**	**Chronic**	

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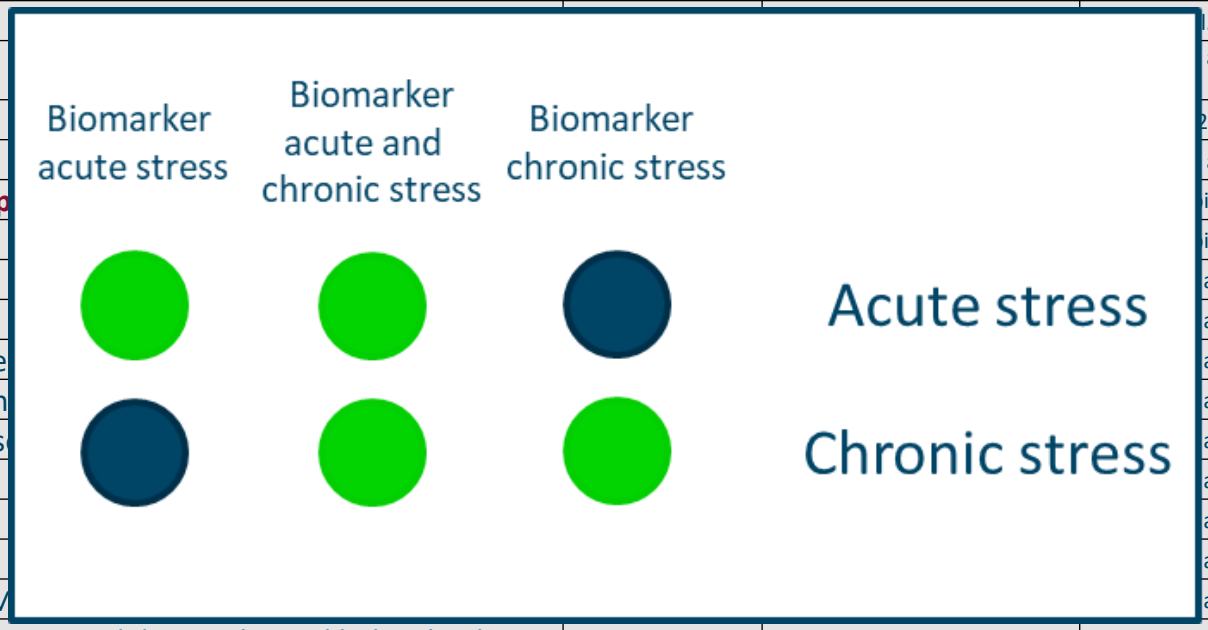

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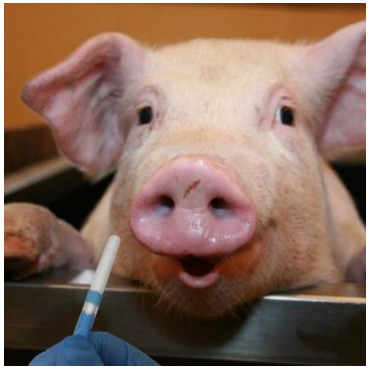
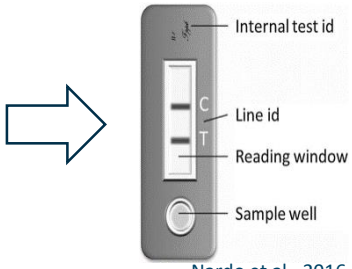
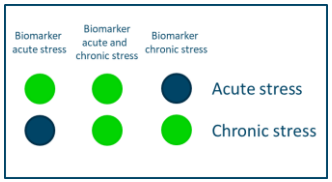
Conclusion

- Saliva is a promising tool to detect chronic stress in piglets
  - Significant differences in salivary proteome profile between the control group and the chronically stressed group
  - Further validation of candidate biomarkers:
    - Different protein detection methods
    - Larger sample size





Nardo et al., 2016



Shirtcliff et al., 2015

Acknowledgment

Applied Veterinary Morphology
University of Antwerp

Prof. C. Casteleyn
Prof. C. Van Ginneken
Prof. S. Van Cruchten
All coworkers from AVM

PPES
Proteinscience, Proteomics & Epigenetic Signaling
University of Antwerp

Prof. X. Van Ostade
Dr. G. Van Raemdonck
All coworkers from PPES

University of Antwerp
University Research Fund (BOF)

fwo Research Foundation Flanders
Opening new horizons

Applied Veterinary
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