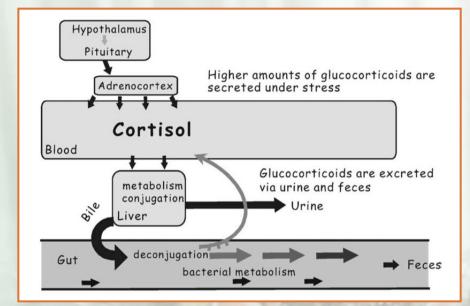
# Determining the adrenocortical activity as a measure of stress in domestic pigs (*Sus scrofa domesticus*) based on salivary and faecal analysis

# Mangwiro N.<sup>1</sup>, Ganswindt A.<sup>2</sup>, Fasina F.O.<sup>3</sup>

<sup>1</sup> Department of Production Animal Studies, University of Pretoria, Onderstepoort 0110, S. Africa, e-mail: mangwiron@yahoo.com; <sup>2</sup> Endocrine Research Laboratory, Department of Anatomy and Physiology, University of Pretoria, Onderstepoort 0110, S. Africa; <sup>3</sup> Department of Veterinary Tropical Diseases, University of Pretoria, Onderstepoort 0110, S. Africa, e-mail: mangwiron@yahoo.com; <sup>2</sup> Endocrine Research Laboratory, Department of Anatomy and Physiology, University of Pretoria, Onderstepoort 0110, S. Africa; <sup>3</sup> Department of Veterinary Tropical Diseases, University of Pretoria, Onderstepoort 0110, S. Africa; <sup>3</sup> Department of Veterinary Tropical Diseases, University of Pretoria, Onderstepoort 0110, S. Africa;

## Introduction

Domestic pigs are subjected to farm management procedures, some of which might be perceived as stressor and therefore may have welfare concerns. Pigs display a response to stressor, which consists of a suite of physiological and behavioural alterations to restore homeostasis. Physiologically, the response (see fig 1) is usually determined using glucocorticoid concentrations (GC) albeit invasively, with the disadvantage of possible handling-induced stress response, an additional factor that may bias the measurement. To date, no non-invasive method exists for determining stress-related responses in pigs have been determined. The aims of the study is to examine the suitability of enzyme-immunoassays (EIAs) for determining GC concentrations in saliva and faeces of domestic pigs using non-invasive methods.



**Fig.1.** Scheme of the secretion, metabolism and excretion of glucocorticoids. (Adapted from Möstl & Palme, 2002)

## Materials and methods

Six pigs were purchased, housed in metabolic cages and randomised for the ACTH challenge (Fig 2). Four (4) received 10µg/kg of Synacthen® (Novartis, South Africa Pty Ltd) each; the remaining 2 pigs received approximately 0.5 ml physiologic saline each. Pigs were monitored hourly and daily and salivary and freshly-voided faecal samples were collected and processed for analyses (Fiess, Heistermann & Hodges 1999; Ganswindt et al. 2012).



**Fig. 2.** Acclimatization, housing and sample collections of experimental pigs



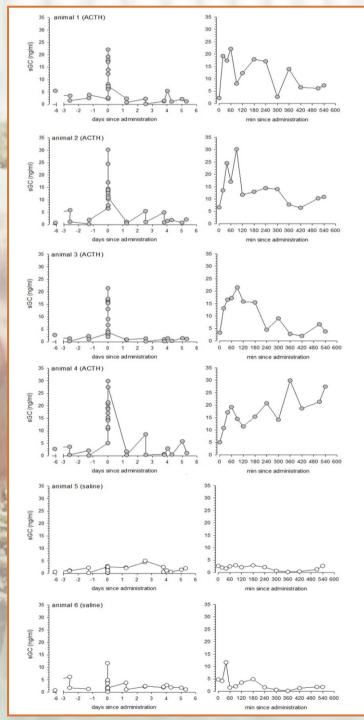
#### **Discussion and conclusion**

This study establishes a non-invasive technique to monitor adrenocortical functions based on measuring concentrations of glucocorticoids or their metabolites in saliva and faeces of domesticated pigs. In this work, both saliva and faeacal samples displayed measurable responses to exogenous ACTH challenge. A 6-fold increase in salivary glucocorticoids levels within 9 hours, an observation that was consistent with other findings (Davies *et al.* 2013)was observed. In this work, the fGCM values rose significantly ranging from 208.90µg/g - 370.90µg/g (Mean±SD 274.90±52.01µg/g DW  $\approx$  15%). It will appear that bacterial enzymes present in the faeces seem to further metabolize FGMs (Möestl, 2014), and the influence of pH on bacterial enzyme activity was also observed.

The ability to reliably assess adrenocortical function in domestic pig using faeces (noninvasive method) now provides a solid basis to further examine endocrine responses to stressful circumstances in the commercially reared pigs.

Potential applications could for instance be the possibility of measuring the potential effect of different rearing practices and housing systems on stress in pig, or more specifically the ability to measure potential variability of fGCM levels in different pig housing set ups. Further, the study clearly underlines the importance of non-invasive hormone measurements as a powerful tool to monitor and provide information about an animal's endocrine status.

Therefore the generated information regarding pigs should help to facilitate further studies which examine endocrine responses to putative stressful circumstances in different pig environments and set ups.





## Results

Baseline salivary glucocorticoid (sGC) concentrations of  $2.38\pm1.83$ ng/ml (mean $\pm$ SEM) increased by six-folds (14.03 $\pm$ 6.83 ng/ml) within 40-90 minutes after administration of synthetic ACTH (P < 0.0001) and the elevated salivary cortisol levels were maintained in test subjects for up to nine hours. Administration of saline resulted in no significant difference in sGC concentration (P = 0.82). Similarly, baseline faecal glucocorticoid metabolite (fGCM) level of 235 $\pm$ 46µg/g rose significantly up to 393 $\pm$ 164µg/g within 36 hours post ACTH administration (P < 0.0001). No significant difference was found between baseline and post saline administration (P = 0.57). Significantly higher sGC concentrations were found in samples collected in the morning compared to those collected in the afternoon, indicating circadian rhythm. In terms of stability of fGCMs post-defecation, respective levels only changed by 4% over the course of 50h (P = 0.76).

**Fig 3.** Individual long-term profiles of salivary glucocorticoid metabolite concentration in gilts (pigs) prior and post administration of either synthetic ACTH (animal 1-4) or saline (animal 5-6). Time point 0 indicated time of administration. The graphs on the left hand side representing the total time interval monitored (-6 to 6 days of administration), whereas the graphs on the right represent the time window of 0 to 10 hour post-injection

#### References

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