

# Validation of Sampling Techniques for Assessing Stress in Pigs by Salivary Cortisol

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## SUMMARY

Salivary cortisol (a stress hormone) is a measure that can be used along with health, behaviour, and productivity as an indication of the stressfulness of a management procedure or system. Saliva sampling is believed to be relatively stress-free, but this needed to be confirmed before using it for tests involving multiple sampling of animals individually or in groups. We sampled individually penned animals repeatedly for 30 minutes, or at 30 minute intervals for 3 hours. Only minor changes in salivary cortisol levels were evident, indicating that multiple sampling could be conducted without inducing significant stress. We also sampled pigs within a group, and determined that repeatedly sampling one pig did not result in an increase in cortisol levels in the other pigs. When conducted according to the protocol used in this study, multiple samplings of saliva can be used to assess cortisol levels in a research barn.

## INTRODUCTION

Cortisol is released into the blood from the outer layer (cortex) of the adrenal gland in response to stress. Thus, we measure cortisol in order to determine the stressfulness of events in pigs' lives, such as regrouping of sows, loading market pigs, or castration of young males. Rather than taking a single sample, we often take multiple samples over time to determine not only the peak level of cortisol, but also how long it remains elevated in response to the stressor. In addition to assessing such short term (acute) stressors, we may also sample cortisol to assess long term (chronic) stressors such as prolonged confinement, overcrowding at feeders, or prolonged social stress. In this case we conduct 'adrenal function' tests in which we monitor release of cortisol over an extended time after first stimulating or blocking the adrenal gland. These tests tell us if the adrenal gland has changed in response to long term stress.

One of the problems associated with using cortisol as an indicator of stress is that we may be measuring the animals' response to our collecting the sample. Laboratory studies typically catheterize animals so that blood can be sampled from outside the room to avoid disturbance. Such techniques are not possible in more applied research settings such as groups of sows or finisher pigs. Blood sampling

will result in a stress response, particularly if the animal is sampled several times. But we have found that cortisol is present in the saliva, at levels proportional to those in the blood, and we feel that sampling saliva may be non-stressful enough to allow us to sample repeatedly without causing a stress response. This series of studies asked the question: Is it possible to repeatedly sample saliva from animals without inducing a significant stress response?

## EXPERIMENTAL PROCEDURES

We sampled pigs that were approximately 70 kg in weight. They were housed either individually or in groups of five. To sample saliva we placed absorbent cotton on the end of a thin metal rod. The cotton was held in place by two rubber stoppers. When this rod was held near the pig's mouth, the pig would chew on the stoppers, allowing the cotton to absorb saliva. The saliva soaked cotton was frozen, later to be thawed, centrifuged, and the cortisol concentrations in the saliva determined by enzyme immunoassay.

*"Pigs can be sampled for salivary cortisol multiple times without inducing a stress response."*

We sampled pigs under three protocols:

- 1) Individually housed pigs (10) were sampled as many times as possible in 30 minutes.
- 2) Individually housed pigs (10) were sampled every 30 minutes for 3 hours.
- 3) One pig in a group of five (10 groups) was sampled every 30 minutes for 3 hours. The other four pigs were sampled at the beginning, and once more at either 30, 60, 120 or 180 minutes.



Sampling the saliva of one pig in a group of five

## RESULTS AND DISCUSSION

For individually penned pigs, sampled repeatedly over 30 minutes, there was a gradual increase in cortisol levels (Figure 1). There was no dramatic increase at any time during the period indicating that the procedure was only mildly stressful.

For individually penned pigs sampled every 30 minutes, there was a slight decline in cortisol levels over time (Figure 2). This could be due to diurnal variation in cortisol concentrations (a gradual decrease during the time of day we sampled), or an adaptation to our presence in the room. From these first two studies it would appear that repeated sampling does not result in a dramatic change in cortisol levels, and thus the technique can be used to sample cortisol levels over time.

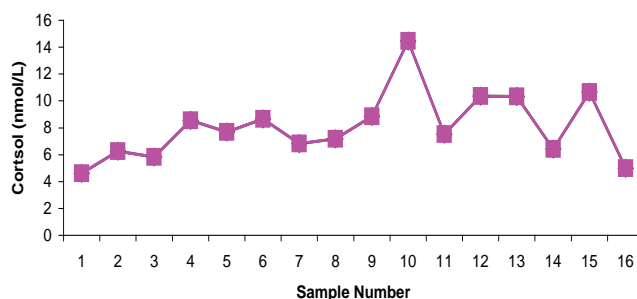
For the group housed animals we did not see a change in cortisol levels over time. Repeatedly sampling one pig in the pen did not affect the cortisol levels of the other pigs. At no time did the cortisol levels of the frequently sampled pig differ from those of the infrequently sampled pigs.

## IMPLICATIONS

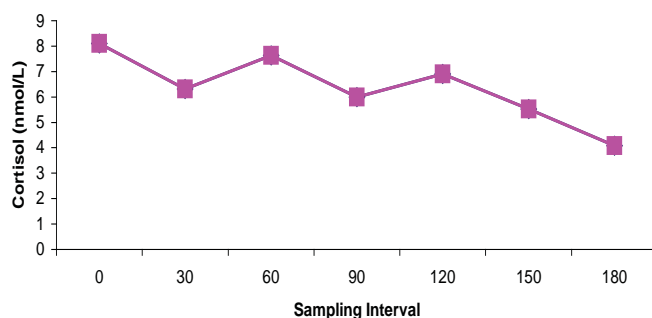
These results indicate that pigs can be sampled for salivary cortisol multiple times without inducing a marked stress response. Thus, we are confident that the cortisol levels obtained are reflective of the environmental conditions (eg. housing or social) rather than due to sampling. In addition, the results demonstrate that several animals in a group can be sampled without distressing the other animals. Our study will continue to develop adrenal function tests which rely on extensive sampling of the animals.

## ACKNOWLEDGEMENTS

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**Figure 1.** Effect of repeatedly sampling the same pig over 30 minutes on cortisol concentration



**Figure 2.** Effect of sampling individually housed pigs every 30 minutes for 3 hours