Investigating novel biomarkers of welfare in swine

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Introduction

There remains increasing concern and debate over the welfare of animals used by humans, particularly those raised for consumption. Consequently, robust measures are required to evaluate the welfare of animals in our care. However, many welfare measures are subjective and relate to what we as humans think is positive or negative for the animal. Within livestock production, identifying objective biomarkers that can inform on animal welfare is important to help support the correct and accurate assessment of welfare with data that can inform public debate and guide management decisions. Biomarkers are objective and quantifiable characteristics of biological processes. Whilst there are many measures taken to evaluate acute experiences that influence animal welfare, such as measurement of cortisol, a hormone that increases in acute stress after aggression. There is currently a lack of reliable biomarkers that can provide information on longer-term welfare in farmed animals. However, the assessment of welfare over a longer period of time is important in the evaluation of husbandry procedures and management practices on animals, such as for example, how different gestation systems influence the welfare of sows, with the stalls vs group-housing debate being one key example: a debate that would have benefitted from improved chronic measures of welfare and stress. Identifying biomarkers in swine could assist in the objective identification and quantification of factors that positively and negatively contribute to an animal's welfare, advance our understanding of animal wellbeing, and provide guidance to improve production systems.

Hair as a medium for evaluating welfare

Cortisol is a hormone of the hypothalamic pituitary adrenal (HPA) axis released in times of stress.. Increased levels of cortisol in saliva and blood have been shown to relate to acute stress in situations such as following weaning, (Erp-van der Kooij et al., 2003) castration (Prunier et al., 2005), transportation (Smiecinska et al., 2011) and mixing with unfamiliar pigs (Erp-van der Kooij et al., 2003). While valuable in certain situation these mediums are unable to inform on long-term stress or support assessments of their overall quality of life. Measurement of hormones within hair has been proposed as a method to evaluate stress over a longer-term. In addition, hair has the benefits of being easily and quickly collected in a non-invasive manner (Fig. 1) compared to other matrices such as blood, and, if properly stored can be saved for months to years for analysis. Hormones are incorporated into the shaft as hair grows and can reflect hormone concentrations over longer periods of time and thus could serve to inform on evaluating emerging issues in the swine industry such as whether group or stall-housed are less stressful for sows. Another hormone of the HPA axis is dehydroepian-drosterone (DHEA). While less researched, DHEA is thought to have roles largely opposing those to cortisol. Studies in humans (Wolkowitz et al., 2001; Rutkowski et al., 2014) and other animals such as cows (Almeida et al., 2008) and mice (Rutkowski et al., 2014) have shown lower levels of DHEA relate to illnesses and disease such as lameness and inflammatory conditions as well as depression and chronic stress. In contrast, higher levels of DHEA have been found to correlate to physical and psychological

Figure 1. Collection of hair from a pig restrained in a weigh crate. Hair is shaved from a consistent area on the rump, and collected on aluminum foil. Collected hair is transferred to a paper envelope for storage.

resilience as well as faster and more successful recovery times in a variety of illness and injuries (Mills et al., 2005; Rutkoswki et al., 2014). Due to the opposing roles of cortisol and DHEA, their ratio has been suggested to be a superior biomarker compared to either analyte separately (Kamin and Kertes, 2016), but studies in this area are extremely limited.

The NSERC Industrial Research Chair program in swine welfare, led by Dr. Yolande Seddon and developed in collaboration with 14 industry partners representing Canadian producers, processors and swine genetics company contains four overarching research goals which focus on emerging questions in swine welfare. The broad objective of Goal 3 is to identify and validate objective biomarkers able to inform on swine welfare, providing monitoring tools that can be used by both the industry and research. Research has been carried out at the Prairie Swine Centre.

One specific objective is to evaluate the use of measuring hormones in swine hair. Over a series of three experiments, the objectives of this goal will evaluate and validate the value of measuring cortisol and DHEA in swine hair as a measure of chronic stress, animal welfare, and of individual stress reactivity in pigs. The results of this work will help to conclude whether hair analysis is a useful tool for use by researchers and industry alike.

Work started (experiment 1) to first refine the laboratory procedures for analysis of hair cortisol and DHEA by evaluating the optimal washing protocol of hair prior and to determine if DHEA could be reliably measured in swine hair. Pig hair varies in cleanliness ranging from visibly clean to hair covered in feces and urine. As feces and urine also contain hormones, external contamination with such matter may influence the hormone concentrations in hair. It is therefore crucial to ensure an effective washing protocol is in place before analysis begins. This study which was published in the fall of 2021 and is available for viewing online (Pollock et al. 2021), showed that DHEA can be measured in swine hair, and a commercially available assay for analysis of DHEA was validated for use in swine. Results also determined that external fecal and urinary contamination influenced the hair cortisol concentrations. Recommendations for the lab analysis of cortisol and DHEA in swine hair were i) to avoid using contaminated hair when possible, and ii) to wash swine hair (visibly contaminated or not) for five three-minute washes using the solvent methanol prior to analysis. This project allowed us to be confident with the laboratory procedure and to proceed to evaluate these hormones as biomarkers of welfare on farm in further studies.

A second experiment explored whether husbandry system could influence the levels of hormones in swine hair. For hormones in hair to be used as a measure of swine welfare, it is important to understand what factors could affect levels. A pig's rearing environment has been shown to influence the release of hormones through HPA activity. One study showed that pigs raised in a conventional fully slatted system had a higher likelihood of developing a blunted circadian rhythm (a natural 24-hour cycle of rising and falling levels of hormones) of cortisol compared to those raised with straw who had a higher likelihood of developing a typical rhythm. However, it is unclear if these changes in the circadian rhythm of cortisol relate to hormone concentrations within the hair and what these changes in the rhythm may mean for the pig. Few studies have investigated the effect of rearing environment on hair cortisol concentrations, with conflicting results. Studies evaluating DHEA are extremely limited, with no studies evaluating the circadian rhythm of DHEA in pigs. To understand if hormones within the hair can inform on swine welfare, it is important to know if there are factors influencing the hormones within the hair. Thus, the objective of this study was to determine if hair cortisol and dehydroepiandrosterone, and their ratios as well as their circadian rhythms differ in pigs raised with straw (Fig. 2) versus those raised in a conventional system. The results of this work will provide useful information to support the correct interpretation of data when measuring hormones in hair from growing swine. This is especially important when considering that husbandry system comparisons are a regularly made with regards to animal welfare.

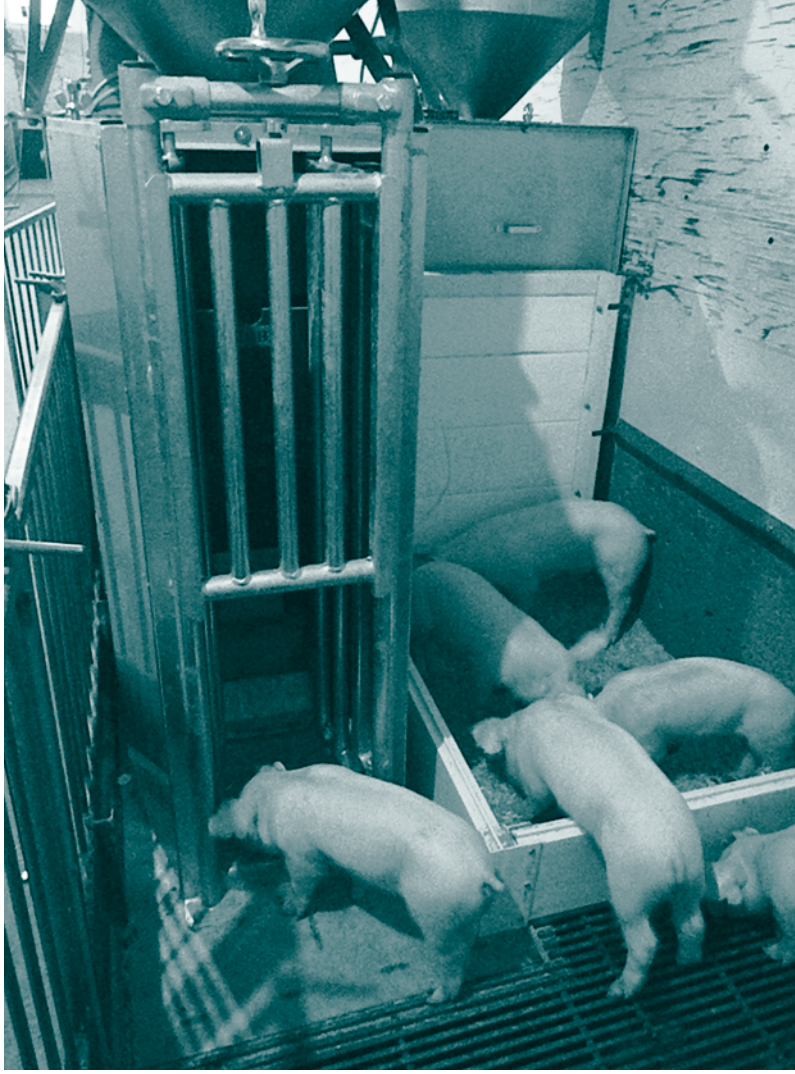

Figure 2. Piglets reared with the provision of straw influenced their levels of hair cortisol and DHEA.

A third study is underway and connects to another research goal within the NSERC IRC in swine welfare (Goal 1) and will determine if targeted management in the early life of the pig that can influence welfare outcomes and stress resilience in the fully-slatted pig resulting in changes in levels of hair cortisol and DHEA. This project provides a platform to further understand how levels of the hormones alter in hair based on welfare

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outcomes and stress resilience of pigs, which is being directly influenced by the experimental treatments. Pigs may experience numerous events throughout their life, many of which occur at a young age, such as processing, castration, weaning and mixing from unfamiliar piglets which may contribute to stress. It may be possible to lessen the overall stress early in life, and in fact positively influence stress resilience and appropriate behaviour development by the addition of positive manipulations such as providing enrichment, increasing space during the pre-weaning period, and providing increased neutral or positive human contact. Early life is also a crucial time for HPA axis development and events early in life may have long term effects on HPA axis development which may affect behaviour and productivity long term. While these manipulations are considered to improve welfare, it is not known if this can be detected within the hair. The results of this study will 1) contribute to understand the ability of measuring cortisol and DHEA in swine hair as a biomarkers of welfare in swine and will be compared to behavioural and productivity measures which is limited in other studies, 2) determine if hair cortisol and DHEA is a reliable and sensitive biomarker of welfare and 3) allow the industry to determine potential benefits of providing early life management strategies such as through the provision of targeted enrichment at different life stages. While enrichment can be difficult to provide in certain barns, providing it early in life may be more feasible.

Finally, we will determine how individual pig traits, stress reactivity, welfare outcomes and productivity measures relate to the levels of cortisol, DHEA and their ratio in the hair of growing swine. The levels of hormones in hair can be highly variable between individuals in a study population, suggesting the reflection of individual differences, which may relate to individual characteristics, like age, stage of growth, stress reactivity, welfare and production performance. Utilizing a database of information accumulated from pigs studied under the experiments of the NSERC IRC in swine welfare, analysis will determine relationships between individual productivity traits (individual growth performance and feed efficiency), welfare outcomes (i.e. was pig tail bitten, lame, in good health), behavioural stress reactivity as quantified through behavioural tests. If successful, this would provide the scientific community and swine industry with an objective measure informing on individual welfare and could aid in genetic selection of resilient pigs.

Implications

If successful, this project will provide the industry and scientific community with a useful, reliable and objective measures of welfare in swine, which could provide information on long-term states, and may also provide information on individual pig stress resilience and performance. The measurement of hormones in hair could in turn can assist in comparing different management systems, individual welfare assessments and genetic selection of stress resilient pigs. Results of ongoing work are expected to be complete by the spring of 2023.

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