Optimising non-invasive cortisol measurement in sheep (Ovis aries)

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Summary

Faecal glucocorticoid metabolite analysis provides a robust non-invasive tool for assessing baseline and acute stress responses of livestock in relation to environmental stressors. In this research project, we quantified faecal cortisol metabolites (FCMs) in sheep using a broad-spectrum polyclonal antibody enzyme-immunoassay. We quantified the underlying variation in FCMs levels in sheep grazing a ‘toxic pasture’ (Biserrula pelecinus) known to cause primary photosensitization (PS)-skin inflammation. Sheep ingesting B. pelecinus had significantly higher FCM levels than controls suggesting a physiological stress response. In conclusion, non-invasive FCM EIA can be applied to assess physiological stress in sheep on farms to assist in addressing health and welfare concerns.

Introduction

Animal welfare is an issue of growing concern in today’s society. One method that is used to assess welfare is stress endocrinology; assessing stress hormones in the body in response to physical (e.g. manual capture) and/or psychological stress (e.g. emotional stress). When an animal is subjected to a stress event, the central nervous system signals the hypothalamus to release corticotrophin-releasing hormone. Corticotrophin-releasing hormone travels to the anterior pituitary causing it to release adrenocorticotrophic hormone (ACTH), this travels to the adrenal cortex, which then produces stress hormone - glucocorticoids (mainly cortisol and/or corticosterone) (Bayazit, 2009). Glucocorticoid production is a vital part of an animal’s stress response as it helps it to cope with the stress event and return its body to a state of homeostasis (back to an unstressed state).

In the past, we have used levels of cortisol in blood plasma as an indicator of stress in livestock; however, this requires taking multiple blood samples via a jugular catheter (Fulkerson and Jamieson, 1982), which is obviously an invasive technique which, ironically, causes the animal more stress. For these reasons, we are starting to look more at non-invasive methods to evaluate stress in livestock, looking at the same stress hormones but without using invasive techniques to measure them.

The primary aim of this research was to validate the R4866 EIA for measuring FCMs in sheep faecal extracts. In Australian farming systems, photosensitisation (PS) is a common problem in sheep production systems due to exposure to toxic plant species that cause either primary or secondary PS (Kessell, Ladmore, & Quinn, 2015). Our study provides an opportunity to apply this innovative non-invasive technique for sheep health and management.

Materials and Methods

All research was undertaken with approval from Charles Sturt University (CSU) Animal Care and Ethics Committee (protocol numbers 15/044, 13/033 and 13/018).

Animals and field study site

All sheep were merino ewes sourced from the CSU flock (n = 100). Sheep were already acclimatised to their paddock when field work began, comprised of naturalized ryegrass pasture containing ryegrass and native grass species with some common pasture weeds. Animals had access to water ad libitum and were intermittently supplemented with oat hay delivered by pickup truck. Apart from occasional hay drops the sheep were left largely undisturbed in the paddock until sampling began in May 2015. Faecal sampling was restricted to one randomly selected section of the paddock, approximately 100 m x 150 m, due to the wide geographical area.

Photosensitivity study - animals and study design

Female crossbred lambs (n = 230) of 7 months of age and mean live weight of 33 kg were sourced from a single local producer. Sheep arrived at CSU farm holding yards by commercial transport on Wednesday 8th July, 2015 and were herded from the transport by dogs as per commercial practice before yarding for allocation to trial plots. Animals were weighed, ear tagged, and had a faecal sample removed directly from the rectum for FCM analysis (day 0). Animals were then allocated to plots and their plot number identified on their dorsal fleece using non-irritant sheep spray paint. All animals were then left overnight in the holding yards, with ample access to fresh feed and water ad libitum. The following day they were yarded again and moved with a small trailer to their treatment pastures, either a non-photosensitising pasture comprising lucerne (Medicago sativa) and subterranean clover (Trifolium subterraneum) dominant or a photosensitising pasture containing a monoculture of Biserrula pelecinus var. ‘Casbah’. Each treatment pasture was a minimum of 0.3 ha in size and contained sufficient biomass of each pasture for feed not to be limiting. Water was provided ad libitum throughout the trial. After 14 d on pasture all animals were again run through the race, weighed, faecal sampled and scored for clinical signs of photosensitivity with a scoring system ranging from 0 (no effect), to 5 (severe).
Faecal cortisol metabolite enzyme immunoassay

Faecal cortisol metabolites were extracted from sheep faecal samples using previously published methods (Möstl et al., 2002). To quantify concentration of FCM in each sample, plates were read at 450 nm (reference 630 nm) on an ELx800 (BioTekTM) microplate reader. The lower limit of detection of the assay was 0.28 ± 0.03 pg/well⁻¹ (n = 7). Intra-assay coefficients of variation were 1.8% and 5.3% for low- and high- percentage bound controls respectively, and inter-assay coefficients of variation were 5.8% and 1.8%, respectively.

Statistical analysis

All FCM data was log transformed to meet the assumptions of homogeneity of variances (Levene’s test) and test for normality. In all studies, ANOVA revealed an overall significant difference in mean FCM concentrations (P < 0.05), therefore post hoc testing was used to determine levels of significant difference between sample groups. Analysis was done in SYSTAT software version 13.0 for all ANOVA and post hoc comparisons. Faecal cortisol metabolite data for all experiments is expressed as ng/g (mean ± S.E.M.) of dry faecal mass.

Post hoc comparisons using Fisher’s Least Significant Difference-Test was utilised to make pairwise comparisons of mean FCM values between the animals on photosensitising or non-photosensitising pastures.

Results and Discussion

Analysis of variance showed a significant difference in mean FCM concentrations of animals grazing the two pasture species on days 0 and 14 (F₃,₅₂ = 4.974, p < 0.005). Post hoc testing using Fisher’s Least-Significant-Difference Test shows the increase in mean FCM concentrations from day 0 to day 14 in sheep grazing biserrula to be highly significant (P < 0.005). There was no comparative increase in sheep grazing lucerne pasture (P > 0.05). Furthermore, comparisons between mean FCM concentrations of sheep grazing biserrula and those grazing lucerne on day 0 were not significantly different (P > 0.05), but on day 14 were (P < 0.05).

In conclusion, development of standard field sample collection devices based on power analysis for number and size of faecal samples required per animal/treatment could also help to minimise error and drive the future of non-invasive field based endocrinology research in livestock (Morgan, et al., 2005).

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References


