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Hair cortisol concentration and adrenal gland weight in healthy and ill cows

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Introduction

It has long been known that certain cattle diseases including tuberculosis, leukosis, metritis and cystic ovarian disease cause an increase in the relative weight of the adrenal glands. Cattle experimentally infected with *Psoroptes ovis* leading to psoroptic mange had significantly greater adrenal gland weights than non-infected controls or infected cattle treated with ivermectin (Blutke et al., 2015). This was thought to be attributable to chronic stress associated with constant pruritus. In humans, chronic stress caused by disorders such as chronic pain (Van Uum et al., 2008) leads to increased cortisol concentrations in hair because during the growth phase, cortisol diffuses passively from the capillaries into the hair shaft (Pragst and Balikowa, 2006) where it is stored (Wosu et al., 2013). Hair cortisol concentration therefore has evolved as a novel biomarker for chronic stress in humans (Russel et al., 2012). There have been several studies on hair cortisol concentrations in healthy and ill cattle (González-de-la-Vara et al., 2011; Comin et al., 2011, 2012, 2013; Peric et al., 2013; Burnett et al., 2014, 2015); ill cattle have higher concentrations than healthy cattle (Comin et al., 2013; Burnett et al., 2015). To the authors' knowledge, there have been no studies comparing adrenal gland weight and hair cortisol concentrations in cattle. The purpose of the present study was therefore to investigate adrenal gland weight and hair cortisol concentrations in relation to the health status of slaughter cows.

Animals, Material and Methods

For this study a total of 142 cows of different breeds, 2 to 16 years of age (median = 4.9 years) was used. Five cows were healthy and 137 had various disorders including lameness (n = 28), soft tissue trauma or fracture (n = 22), obstetrical problems (n = 17), downer cow syndrome (n = 16), gastrointestinal disease (n = 11), mastitis (n = 5), neoplasia (n = 4), bronchopneumonia (n = 3), peritonitis (n = 2) or a combination of multiple disorders (n = 27). Two cows had no diagnosis. Sixty

cows had a known duration of illness and were divided into groups A (acutely ill, n = 30) and B (chronically ill, n = 30). Cows of group A underwent emergency slaughter because of acute illness of no more than 3 days duration (injury, uterine torsion, ileus). Cows of group B had a history of illness for at least 4 weeks and were slaughtered also. The findings of the two groups were compared. After slaughter, all internal organs including the adrenal glands and the claws were examined and the carcasses weighed. The adrenal glands were removed and weighed individually after removing all attached non-adrenal gland tissues. The hair in a 5 by 5 cm area on the neck was removed using electric clippers, dried, wrapped in tin foil and stored at room temperature until analysis. Liquid chromatography tandem mass spectrometry (LC-MS/MS) was used for measurement of the hair cortisol concentration as described in detail (Binz et al., 2016). Data were tested for normality using the Kolmogorov-Smirnov test, and mean and standard deviation were calculated for normal data and the median for non-normal data. Adrenal gland weights of groups A (acutely ill) and B (chronically ill) were compared using a t test, and cortisol concentrations were compared using the Mann-Whitney U test. These comparisons were made for each illness using the Kruskal-Wallis H test and Mann-Whitney U test with Bonferroni correction for post hoc analysis. Pearson correlation coefficients between adrenal gland weights and hair cortisol concentrations were calculated.

Results

The mean weight of the left adrenal gland was 21.1 ± 6.0 g (range, 8 to 41 g) and the weight of the right gland was 18.8 ± 5.7 g (range, 7 to 38 g). Adrenal weights were positively correlated with age ($r = 0.46$, $P < 0.01$) and carcass weight ($r = 0.38$, $P < 0.01$). The weights of left and right adrenal glands of acutely and chronically ill cows did not differ significantly (Tab. 1). Chronically ill cows had significantly higher hair cortisol concentrations (median = 1.37 pg/mg) than acutely ill cows (median = 0.56 pg/mg, Tab. 1, Fig. 1). Hair cortisol concen-

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Table 1: Adrenal gland weights (mean ± SD, range) and hair cortisol concentrations (median, range) of acutely (group A) and chronically ill cows (group B).

Adrenal gland	Group A (n = 30)	Group B (n = 30)
Weight of left gland (g)	19.8 ± 5.5 (12.3 – 36.0)	22.8 ± 6.2 (11.7 – 38.6)
Weight of right gland (g)	17.7 ± 5.4 (9.9 – 33.8)	20.1 ± 5.8 (10.4 – 37.9)
Hair cortisol (pg/mg)	0.56 (0.13 – 4.39)	1.37** (0.51 – 9.85)

** Groups differ (P < 0.01)

tration differed among the disease groups (P < 0.01); cows with lameness (median = 1.20 pg/mg, 0.05–6.05 pg/mg), multiple disorders (1.19 pg/mg, 0.14–28.51 pg/mg), gastrointestinal disease (1.13 pg/mg, 0.16–4.60 pg/mg), pneumonia/peritonitis (1.03 pg/mg, 0.51–3.31 pg/mg) and mastitis (0.59 pg/mg, 0.46–1.24 pg/mg) had greater concentrations, and cows with neoplasia (0.25 pg/mg, 0.22–0.31 pg/mg) had lower concentrations than healthy cows (0.43 pg/mg, 0.32–0.55 pg/mg; P < 0.05). The hair cortisol concentration of cows with soft tissue trauma or fracture (0.58 pg/mg, 0.16–4.39 pg/mg), obstetrical disorders (0.62 pg/mg, 0.13–9.09 pg/mg) and downer cow syndrome (0.74, 0.25–9.62 pg/mg) did not differ significantly from those of the healthy cows (P > 0.05). There were no significant correlations between adrenal gland weights and hair cortisol concentrations.

Four cows with multiple disorders had hair cortisol concentrations exceeding 9.0 pg/mg and 3 of them had above average adrenal weights.

Discussion

The mean weights of the left and right adrenal glands were considerably greater than the respective weights of 14.2 and 12.8 g reported in an anatomy text (Böhme, 2003). In contrast to the study of Blutke et al. (2015) in *Psoroptes*-infested cattle, chronically ill cows had not significantly greater adrenal gland weights than acutely ill cows. The median hair cortisol concentration was 0.85 pg/mg hair with a range of 0.05 to 28.51 pg/mg. In other studies, the concentration measured in hair varied from 2.1 to 5.38 pg/mg (Comin et al., 2011, 2012, 2013; Peric et al., 2013). Overall, hair cortisol concentrations measured in the present study were lower than those of comparable studies and the most likely reason for this are methodical differences. We used a technique that combined liquid chromatography and mass spectrometry (LC-MS/MS) for cortisol measurement whereas others used a radio immunoassay (González-de-la-Vara et al., 2011; Comin et al., 2011, 2012, 2013; Peric et al., 2013), an ELISA (Burnett et al., 2014) or enzyme immunoassay (Burnett et al., 2015). Mass spectrometry is considered the gold standard for hair analysis because it is more sensitive and more specific than other

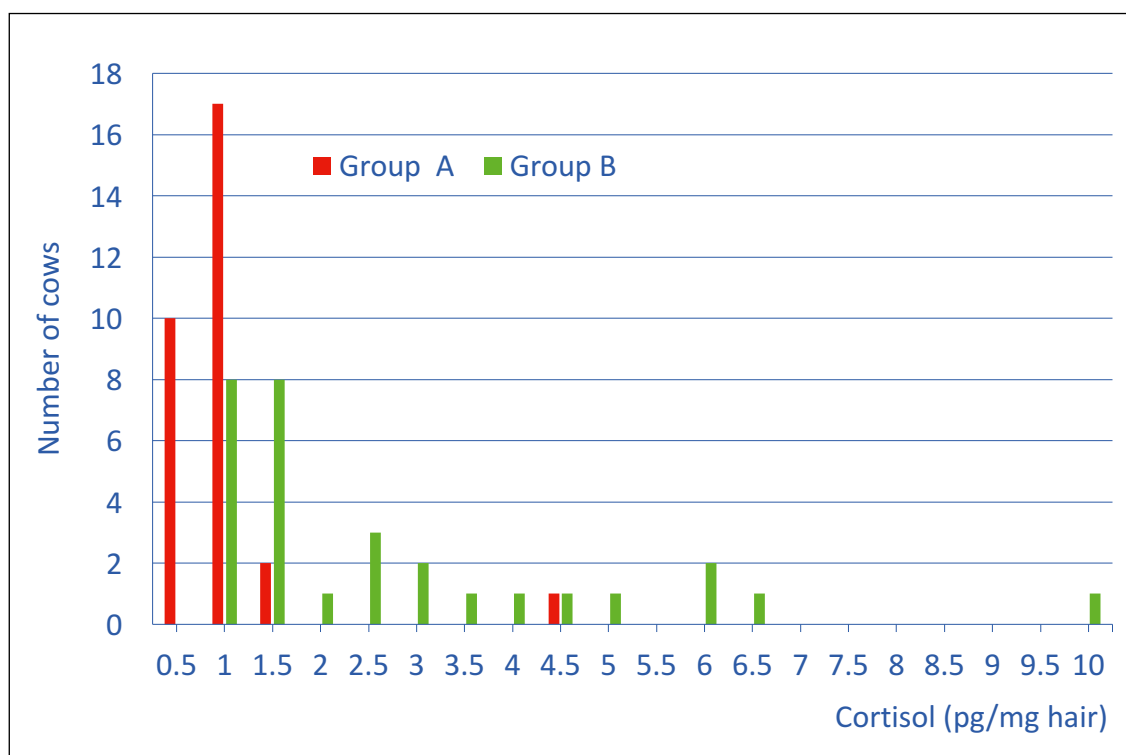


Figure 1: Frequency distribution of hair cortisol concentration in 30 acutely ill and 30 chronically ill cows.

methods (Gow et al., 2010). Immunoassay techniques generally measure cortisol concentrations that are 2.5 to 10-fold higher than those measured by LC-MS/MS methods because of assay-dependent cross-reactivity with other steroid hormones (Russell et al., 2015). The method used in the present study had a recovery of about 85%.

Chronically ill cows had higher hair cortisol concentrations than acutely ill cows, which was in agreement with findings of another study (Burnett et al., 2015). There was no correlation overall between hair cortisol concentration and adrenal gland weight. However, more studies are necessary to elucidate the relationship between adrenal gland weight and hair cortisol concentration in chronically ill cows compared to healthy cows.

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