Wien. Tierärztl. Mschr. **86** (1999), 237 - 241
Verlag OSTAG Wien

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Measurement of faecal cortisol metabolites in ruminants: a non-invasive parameter of adrenocortical function

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received for publication January 15th, 1999

Keywords: blood, faeces, cortisol metabolites, non-invasive, stress, cattle, sheep, enzyme immunoassay.

Summary

In mammals under stress glucocorticoids are secreted by the adrenal cortex. Only recently an enzyme immunoassay for 11,17-dioxoandrostanes has been established to allow for a determination of faecal cortisol metabolites in ruminants, thus providing the basis for a non-invasive evaluation of adrenocortical activity.

The aim of this study was to test the biological relevance of this method in ruminants (cattle, sheep) by stimulating or suppressing cortisol release by the adrenal cortex. Adrenocorticotropic hormone (ACTH: cattle 1 mg, sheep 0.5 mg) and dexamethasone (30 mg or 4 mg) were injected i.v. successively into six animals (three of each sex) of both species. In addition, different amounts of ACTH (0.25 mg, 0.5 mg and 3 doses of 1 mg each, 2 hours apart) were injected into two cows each. Concentrations of cortisol in blood, sampled via a permanent catheter, and cortisol metabolites in faeces were determined. Variability among animals concerning both basal and peak values in blood and faeces were observed. Concentrations of 11,17-dioxoandrostanes in faeces paralleled those of cortisol in blood with a delay of about 10 hours. ACTH or dexamethasone injections resulted in an increase (by 2.3 to 24 times) or decrease (8 % to 27 % of basal levels), respectively, of 11,17-dioxoandrostanes concentrations.

Therefore, measuring faecal 11,17-dioxoandrostanes can be implemented as a non-invasive tool for monitoring adrenocortical function in ruminants.

Schlüsselwörter: Blut, Kot, Kortisolmetaboliten, nicht invasiv, Streß, Rind, Schaf, Enzymimmunoassay.

Zusammenfassung

Bestimmung der Konzentration von Kortisolmetaboliten im Kot von Wiederkäuern als nicht invasiver Parameter für die Nebennierenrindenaktivität

Glukokortikoide werden in Belastungssituationen von der Nebennierenrinde vermehrt ins Blut abgegeben. Ein vor kurzem entwickelter Enzymimmunoassay (EIA) erlaubt die Messung von Kortisolmetaboliten (11,17-Dioxoandrostane) im Kot.

Ziel dieser Arbeit war es, die biologische Relevanz dieser Bestimmungsmethode beim Wiederkäuer zu überprüfen. Dazu wurde bei jeweils 3 männlichen und 3 weiblichen Rindern bzw. Schafen die Nebennierenrindenaktivität durch ACTH (1,0 mg bzw. 0,5 mg i.v.) stimuliert bzw. durch Dexamethason (30 mg bzw. 4 mg i.v.) unterdrückt. Bei Kühen wurden zusätzlich unterschiedliche Mengen von ACTH (0,25; 0,5; 3x1 mg) an je 2 Tiere verabreicht. Über einen Zeitraum von 7 Tagen wurden Blut- und Kotproben gesammelt. Die Konzentration von Kortisol im Blut (mittels Katheter gewonnen) bzw. der 11,17-Dioxoandrostane im Kot wurde mittels EIA bestimmt. Es wurden individuelle Unterschiede sowohl bei den Basis- als auch bei den Spitzenwerten im Blut und Kot bei den einzelnen Tieren beobachtet. Die Verlaufskurve der Kortisolmetabolitenkonzentration im Kot folgte bei allen Versuchstieren mit einer zeitlichen Verzögerung von ca. 10 Stunden der Verlaufskurve der Kortisolkonzentration im Blut. Eine ACTH- bzw. Dexamethasonapplikation führte zu einem Ansteigen (2,3 bis 24fach) oder Absinken (8 % bis 27 % der Basalwerte) der Konzentration von 11,17-Dioxoandrostanen im Kot.

Diese Studie zeigt, daß die Bestimmung der Kortisolmetabolitenkonzentration im Kot ein neues, nicht-invasives Hilfsmittel zur Beurteilung von Belastungen beim Wiederkäuer ist.

Introduction

A physiological response to stress involves the release of pituitary-derived adrenocorticotropic hormone (ACTH) and subsequent secretion of glucocorticoids by the adrenal cortex (AXELROD and REISINE, 1984). Although caution is advised, since glucocorticoid determination is not sensitive to every type of stressor, and since there is considerable inter-animal variability in biological responses, blood cortisol analysis has proven useful as an indicator of stress under a variety of conditions and in a variety of species including wildlife (e.g., BROOM and JOHNSON, 1993; MORTON et

al., 1995; TERLOUW et al., 1997). However, as blood sample collection itself causes stress and is dangerous or even impossible in some zoo and wildlife species, feedback-free methods for the determination of glucocorticoids (or their metabolites) are a prerequisite for assessing stress in these animals. Collecting saliva includes some handling of the animal and urine samples are difficult to obtain and, therefore, impractical under field conditions. Above all, faecal samples offer the advantage that they can be collected easily without any need to handle the animal.

The analysis of faecal oestrogens and progestagens for non-invasive reproductive monitoring is an established technique used in an increasing number of laboratories (SCHWARZENBERGER et al., 1996). In contrast to these steroids, glucocorticoids are metabolized extensively (BROWNIE, 1992; MACDONALD et al., 1983). Recent infusion studies (1 g cortisol or ^{14}C-cortisol) in sheep (PALME and MÖSTL, 1997) showed that cortisol and tetrahydrocortisol could not be detected in the faeces. Almost all available cortisol immunoassays are specific and, therefore, do not show significant cross-reactions with the faecal corticoid metabolites in ruminants. On the basis of characterized faecal metabolites of infused ^{14}C-cortisol in sheep, an enzyme immunoassay (EIA) for 11,17-dioxoandrostanes (11-oxoaetiocholanolone-EIA) was established by PALME and MÖSTL (1997). Delay times of faecal excretion (PALME et al., 1996) must be taken into account, which allow for the monitoring of stressful events which occurred during a species-specific period before the sampling. By dampening minor short term fluctuations in cortisol secretion, faecal metabolite analysis may improve the ability to distinguish between normal pulsatile dynamics and genuine physiological responses to externally mediated (i.e., stressful) events.

The aim of this study was to test the biological relevance of this newly established method. Similarities in the pattern of cortisol release following ACTH administration and physiological stressors were observed in animals (e.g., ESSAWY et al., 1989; FULKERSON and JAMIESON, 1982; SHAYANFAR et al., 1975; VEISSIER and LE NEINDRE, 1988). Therefore, either stimulation of the adrenal cortex using ACTH or suppression of the cortisol production using dexamethasone was performed in ruminants (cattle, sheep) to determine excretion patterns of faecal cortisol metabolites following these administrations.

Material and Methods

Administrations

$ACTH_{(1-24)}$ (Synacthen®, CIBA-Geigy, Switzerland) was administered (cattle: 1 mg; sheep: 0.5 mg) by a single i.v. injection. In a second experiment dexamethasone-21-disodiumphosphate (Dexa-"TAD"®, Ogris Pharma, Austria) was given in a high therapeutical dosis (cattle: 30 mg, sheep: 4 mg). Both drugs were given to six (three of each sex) healthy animals of each species (cattle, sheep) from the research farm of the Veterinary University of Vienna. In one bull the ACTH challenge could not be performed due to illness at that time. In a third experiment different amounts of ACTH (0.25 mg, 0.5 mg and 3 doses of 1 mg each, 2 hours apart) were injected i.v. into two cows each. All injections were done through the sampling catheter. Cattle were mainly Austrian Fleckvieh (including one Austrian Brown and one Black and White) and aged from 1 to 10 years. Sheep were mainly of the Austrian breed "Steinschaf" (including two East Friesian Milk Sheep) and between 0.3 and 3.3 years of age.

Sampling and determination of steroids

To minimize stress during blood sampling, a permanent catheter (Secalon Universal Catheter 16G 1.8 mm x 650 mm; Co. Ohmeda, Swindou, UK) was inserted (ROBIA, 1998) into the V. auricularis (only cows) or the V. jugularis (bulls and sheep). The catheter was placed 3 days before the sampling started in order to avoid any stress by the procedure itself. Blood was taken twice daily from day -2 to day +5 via the catheter. Following ACTH administration, additional samples were obtained 30, 60, 90, 120, 360 and 540 min after injection. Blood samples were centrifuged and the plasma stored at -20 °C until analysis. Cortisol concentrations were determined by an EIA after extraction with diethylether (PALME and MÖSTL, 1997).

All voided faecal samples were collected on the day of the administration and on the following 2 days. In addition, about four to eight samples from another 2 days before and after this period were taken. Faeces were stored at -20 °C until analysis, which was performed according to PALME and MÖSTL (1997). Briefly, portions of 0.5 g of each sample were extracted with 5 ml of methanol (80 %). After shaking (30 min) and centrifugation (2,500 g; 15 min) the amounts of cortisol metabolites (11,17-dioxoandrostanes) in the supernatant were directly determined by a group specific enzyme immunoassay (11-oxoaetiocholanolone-EIA). The intra- (inter-) assay coefficients of variation for a low and high concentration pool were 10.1 % (13.2 %) and 11.9 % (14.3 %), respectively. The limit of detection of this method was 5 nmol/kg faeces.

Statistical analysis

Basal levels of steroids in blood and faeces were defined as the median of the concentration of all samples collected before administration. Values of all faecal samples of a species and administration type were grouped according to time (intervalls of 6 hours before and after every half day, see Fig. 2) . These data were analyzed by the Mann-Whitney U-Test to check for significant differences between the intervals. In addition, in ACTH administrations in cattle, correlations (Pearson Product Moment Correlation) between plasma cortisol and faecal metabolites (peak values, increase above basal values and time course pattern) were determined. All statistical analysis was performed using Sigma-Stat© (SPSS Inc., Germany).

Results

Inter-animal variability concerning both basal and peak values in blood and faeces was observed. Before administration basal blood cortisol levels ranged from 1.1 to 20.2 (median: 2.4) nmol/l in cattle and from 1.6 to 17.8 (median: 6.9) nmol/l in sheep. Basal levels of 11,17-dioxoandrostanes ranged from 34 to 445 (median: 93) nmol/kg faeces and from 93 to 1,031 (median: 312) nmol/kg faeces, respectively.

After administration in both species, concentrations of faecal cortisol metabolites paralleled those of blood cortisol, showing a delay of approximately 10 hours (Fig. 1). Following

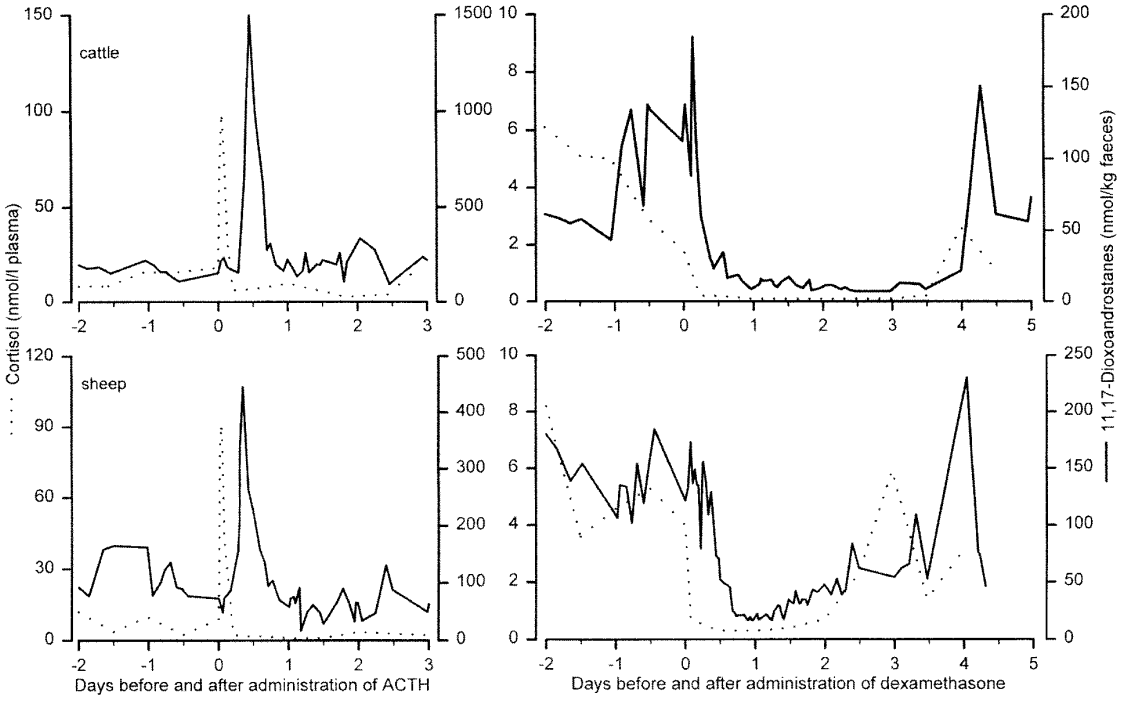

Fig. 1: Concentrations of cortisol in blood (nmol/l) and cortisol metabolites (11,17-dioxoandrostanes) in faeces (nmol/kg) before and after administration of ACTH (1 mg or 0.5 mg, respectively; left panels) and dexamethasone (30 mg or 4 mg, respectively; right panels) in a cow (upper panels) and a sheep (lower panels)

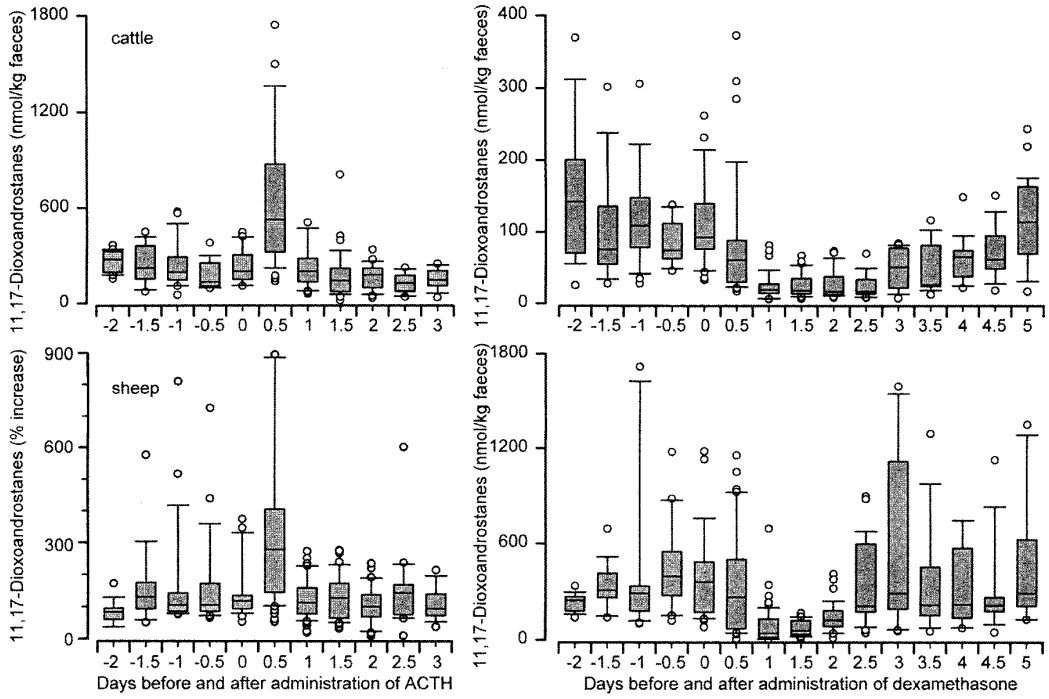

Fig. 2: Boxplot of grouped concentrations (intervals of ± 0.25 days) of cortisol metabolites (11,17-dioxoandrostanes) in faeces (nmol/kg) before and after administration of ACTH (1 mg or 0.5 mg, respectively; left panels) and dexamethasone (30 mg or 4 mg, respectively; right panels) in cattle (upper panels) and sheep (lower panels); note that in the case of ACTH administrations in sheep concentrations are expressed as percentage (%) of basal levels

ACTH challenge, plasma cortisol concentrations peaked after 1 - 5 hours (median: 1.5 hours) and returned to basal levels within 5.0 to 9.1 hours (or 13 hours following administration of 3 doses of 1 mg ACTH). In cattle, after administration of a higher dose, peak concentrations were reached later ($r = 0.9$; $p < 0.0002$). Maximal cortisol concentrations ranged from 32.2 to 155.5 (median: 74) nmol/l in all animals. Increases, expressed as percentage above basal levels, ranged from 640 to 6,370 % (median: 2,000 %). Maximal concentrations of faecal cortisol metabolites were reached about 10 hours (6.0 to 18.7 hours) after blood cortisol peaked, and returned to basal levels between 18 to 44 hours following ACTH injection. Peak concentrations in cattle and sheep ranged from 745 to 1,954 (median: 1,118) and from 445 to 3,622 (median: 1,719) nmol/kg faeces, respectively. This reflected an increase of 230 % - 2,440 % (590 %) and 230 % - 1,510 % (510 %), respectively. In cattle the grouped concentrations of all samples voided between 6 hours and 18 hours following ACTH injection were significantly higher ($p < 0.001$) than at any other interval (Fig. 2). In sheep, due to high inter-animal variability in basal levels, the same pattern was only found after expressing each concentration as a percentage of basal values (Fig. 2).

In cattle, no correlation was found between administered dose and maximal concentrations in blood or faeces and dose and percentage of the increase in blood. However, dose and the increase (%) of faecal cortisol metabolites were correlated ($r = 0.77$; $p = 0.006$). In addition, basal and peak values, both in blood and faeces were correlated ($r = 0.87$; $p = 0.0005$ and $r = 0.76$; $p = 0.006$, respectively). A correlation was found between maximal concentrations in blood and faeces ($r = 0.74$; $p = 0.009$), but not between basal values.

Following dexamethasone injection, plasma cortisol values around or below the detection limit (0.3 nmol/l) were measured 12 to 24 hours afterwards. Basal levels were reached again after 2 or more days (in 3 cattle this was not monitored during the sampling period). Minimal concentrations (8 % to 27 %; median: 14 % of basal levels) of faecal cortisol metabolites were found about 23 hours (16.3 to 36 hours) after injection (Figs. 1 and 2). In most of these administrations a small transient increase in concentrations could be monitored about 8 to 10 hours following injection. Basal levels were reached again after 4 or more days in cattle and after 2.3 to 5.3 (median 3.2) days in sheep. In both species, grouped faecal samples from the intervals (± 0.25 days) around 1, 1.5 and 2 days (in cattle also 2.5 days) showed lower ($p < 0.001$) concentrations of 11,17-dioxoandrostanes than those of the other days (Fig. 2). In sheep the first two of these intervals were the lowest ($p = 0.003$). Only in cattle, values of intervals around day 0.5 and from days 3 to 4.5 were also of lower ($p < 0.01$) concentration than basal values.

Discussion

Injections of ACTH have been used frequently in ruminants to assess adrenal function during various physiological states (ESSAWY et al., 1989; FULKERSON and JAMIESON, 1982; LAY et al., 1996; VEISSIER and LE NEINDRE, 1988; VERKERK et al., 1994). In our study pharmacological stimulation and suppression of cortisol release by the adrenal gland was used to evaluate the biological relevance of a recently established method for measuring faecal cortisol metabolites (PALME and MÖSTL, 1997). Therefore patterns of faecal 11,17-dioxoandrostane concentrations in relation to plasma cortisol were monitored.

Mean basal values of plasma cortisol were in the lower range of reported values (e.g., ALAM et al., 1986; ESSAWY et al., 1989; FULKERSON and JAMIESON, 1982; LAY et al., 1996; SIXT et al., 1997; TOUTAIN et al., 1982). This may be caused by both the specificity of the EIA used and less stressful blood sampling, as indwelling catheters were used and the animals were accustomed to the handling. Patterns of plasma cortisol concentrations after ACTH or dexamethasone administration were similiar to comparable studies reported in the literature (e.g., ALAM et al., 1986; ESSAWY et al., 1989; FULKERSON and JAMIESON, 1982; TOUTAIN et al., 1982; VEISSIER and LE NEINDRE, 1988). Concentrations of faecal cortisol metabolites paralleled those of blood cortisol, showing a delay of approximately 10 hours. The delay times of peak concentrations of faecal 11,17-dioxoandrostanes were in the range of those observed after infusion of radiolabelled ^{14}C-steroids (PALME et al., 1996).

Inter-animal variation in the concentration of faecal cortisol metabolites was large, as was reported for cortisol in blood (ALAM et al., 1986; ESSAWY et al., 1989; FULKERSON and JAMIESON, 1982; SHAYANFAR et al., 1975). Thus, for experiments evaluating stress responses either large numbers of animals are needed or statistical procedures must be used so that an animal acts as its own control, reducing the influence of individual variation.

No correlation between peak plasma cortisol concentration and dose could be found, probably due to the high pharmacological doses used (ALAM et al., 1986). However, such a correlation was measured for the maximal increase (%) of faecal cortisol metabolites above basal levels. Therefore, faecal concentrations of cortisol metabolites reflect the amounts of excreted and, therefore, produced cortisol better than a blood concentration, which changes quickly. This underlines the usefulness of measuring faecal 11,17-dioxoandrostanes as an indicator of amounts of cortisol released. In this case frequent blood sampling to determine the integrated adrenal response (calculated from the area under the curve described by cortisol values), which is reported to be dose related (FRIEND et al., 1977; LAY et al., 1996; VERKERK et al., 1994), could be avoided. Lower doses of ACTH to determine the sensitivity (smallest plasma cortisol peak which is reflected in the faeces) and the influence of age, gender and breed on faecal cortisol metabolites need further investigation.

A transient increase in concentrations of faecal 11,17-dioxoandrostanes was observed in some animals after dexamethasone injection. This probably reflected a similar rise in blood cortisol concentration, possibly related to side effects observed after drug administration as described by TOUTAIN et al. (1982). Suppression of adrenocortical activity was more pronounced in cattle than in sheep, indicating a higher sensitivity (on a body weight basis) of this species to dexamethasone.

In conclusion, changes in plasma cortisol were well reflected by faecal cortisol metabolites in cattle and sheep and would, therefore, most likely react similarly in other ruminants. Attempts to understand physiological responses of animals to stress are often confounded by the inva-

siveness of the sampling methods (e.g. blood sampling) and the need to restrain animals during such procedures. Therefore measuring faecal 11,17-dioxoandrostanes as an indicator of adrenocortical activity in farm, zoo and wildlife ruminants offers the advantage of a simple sampling technique, which does not interfere with the results of the experiment and enables even long term longitudinal studies. Thus, the established method should be a valuable tool in a variety of research fields such as animal welfare (handling, housing and transportation) but also in ethological and environmental studies.

Acknowledgements

The authors thank Mrs. M. Stark for her excellent assistance in the laboratory, and the research farm of the Veterinary University of Vienna (head: Dr. M. Dobretsberger) for providing the animals. The financial support of the Austrian Science Fund (FWF; Proj. No.: P12376-Med) is gratefully acknowledged.

Literature

ALAM, M. G. S., DOBSON, H., FITZPATRICK, R. J. (1986): Endocrine response to different doses of ACTH in cows. Brit. Vet. J. **142**, 239 - 245.

AXELROD, J., REISINE, T. D. (1984): Stress hormones: their interaction and regulation. Science. **224**, 452 - 459.

BROOM, D. M., JOHNSON, K. G. (1993): Stress and animal welfare. Chapman and Hall, London, p. 87 - 144.

BROWNIE, A. C. (1992): The metabolism of adrenal cortical steroids. In: JAMES, V. H. T. (ed.): The adrenal gland. Raven Press, New York, p. 209 - 224.

ESSAWY, S. A., BENHAJ, K. M., COOKE, R. G., DOBSON, H. (1989): Effect of dose and route of administration of $ACTH_{1-24}$ on plasma cortisol concentrations in ewes. J. Vet. Pharm. Therap. **12**, 302 - 306.

FRIEND, T. H., POLAN, C. E., GWAZDAUSKAS, F. C., HEALD, C. W. (1977): Adrenal glucocorticoid response to exogenous adrenocorticotropin mediated by density and social disruption in lactating cows. J. Dairy Sci. **60**, 1958 - 1963.

FULKERSON, W. J., JAMIESON, P. A. (1982): Pattern of cortisol release in sheep following administration of synthetic ACTH or imposition of various stressor agents. Austr. J. Biol. Sci. **35**, 215 - 222.

LAY, D. C. jr., FRIEND, T. H., RANDEL, R. D., JENKINS, O .C., NEUENDORFF, D. A., KAPP, G. M., BUSHONG, D. M. (1996): Adrenocorticotropic hormone dose response and some physiological effects of transportation on pregnant Brahman cattle. J. Anim. Sci. **74**, 1806 - 1811.

MACDONALD, I. A., BOKKENHEUSER, V. D., WINTER, J., MCLERNON, A. M., MOSBACH, E. H. (1983): Degradation of steroids in the human gut. J. Lip. Res. **24**, 675 - 700.

MORTON, D. J., ANDERSON, E., FOGGIN, C. M., KOCK, M. D., TIRAN, E. P. (1995): Plasma cortisol as an indicator of stress due to capture and translocation in wildlife species. Vet. Rec. **136**, 60 - 63.

PALME, R., MÖSTL, E. (1997): Measurement of cortisol metabolites in faeces of sheep as a parameter of cortisol concentration in blood. Int. J. Mammal. Biol. **62** (Suppl II), 192 - 197.

PALME, R., FISCHER, P., SCHILDORFER, H., ISMAIL, M. N. (1996): Excretion of infused ^{14}C-steroid hormones via faeces and urine in domestic livestock. Anim. Reprod. Sci. **43**, 43 - 63.

ROBIA, C. (1998): Bestimmung von Cortisolmetaboliten im Kot als Parameter für die Cortisolkonzentration im Blut bei Wiederkäuern. Diss., Vet. Med. Univ. Wien.

SCHWARZENBERGER, F., MÖSTL, E., PALME, R., BAMBERG, E. (1996): Faecal steroid analysis for non-invasive monitoring of reproductive status in farm, wild and zoo animals. Anim. Reprod. Sci. **42**, 515 - 526.

SHAYANFAR, F., HEAD, H. H., WILCOX, C. J., THATCHER, W. W. (1975): Adrenal responsiveness in lactating Holstein cows. J. Dairy Sci. **58**, 870 - 878.

SIXT, A., STANEK, Ch., MÖSTL, E. (1997): The influence of different claw trimming methods on plasma cortisol levels in dairy cows. Wien. Tierärztl. Mschr. **84**, 181 - 188.

TERLOUW, E. M. C., SCHOUTEN, W. G. P., LADEWIG, J. (1997): Physiology. In: APPLEBY, M. C., HUGHES, B. O. (eds.): Animal Welfare. CAB International, University Press, Cambridge, p. 143 - 158.

TOUTAIN, P. L., BRANDON, R. A., ALVINERIE, M., GARCIA-VILLAR, R., RUCKEBUSCH, Y. (1982): Dexamethasone in cattle: pharmacokinetics and action on the adrenal gland. J. Vet. Pharmacol. Therap. **5**, 33 - 43.

VEISSIER, I., LE NEINDRE, P. (1988): Cortisol responses to physical and pharmacological stimuli in heifers. Reprod. Nutr. Develop. **28**, 553 - 562.

VERKERK, G. A., MACMILLAN, K. L., MCLEAY, L. M. (1994): Adrenal cortex response to adrenocorticotropic hormone in dairy cattle. Dom. Anim. Endocrin. **11**, 115 - 123.

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