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## Excretion of catecholamines (adrenaline and noradrenaline) in domestic livestock

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### Summary

The adrenals play a key-role in the hormonal stress reactions and non-invasive methods are favoured for evaluating their activity, as blood sampling may cause an increase in these hormones. Above all, faeces offer the advantage that they can be collected easily and feedback free. Measurement of faecal glucocorticoid metabolites is used in an increasing number of species. However, literature concerning the excretion of catecholamines (CA) via the faeces is not available. The aim of this study was to gain basic information about the excretion of CA in sheep and swine. In addition the main faecal metabolites were characterised. This should clarify, whether or not methods for the determination of some of the relevant faecal metabolites can be developed to allow a non-invasive evaluation of sympathoadrenal activity during disturbances. Therefore, the percentage and the time course of the excreted radioactivity in urine and faeces were determined after intravenous injection of <sup>3</sup>H-adrenaline (<sup>3</sup>H-A) and <sup>3</sup>H-noradrenaline (<sup>3</sup>H-NA; 3.7 MBq each) in 4 sheep (2 of each sex; a total of 8 administrations) and 2 sows. Urinary and faecal samples were collected and radioactivity was measured by liquid scintillation counting. Administered CA were excreted mainly in the urine (>96 %). The highest radioactivity in the urine (per litre) of all animals was observed in one of the first 2 samples following injection, whereas in faeces it was measured with a delay of about 12 h in sheep and 48 h in sows. Thereafter, the radioactivity declined slowly and reached background levels within 4 weeks. In the faeces mainly metabolites of CA were present. However, due to the low amounts of faecal metabolites of the injected CA a quantitative measurement in sheep and sows seems to be not possible.

### Zusammenfassung

#### Ausscheidung von Katecholaminen (Adrenalin und Noradrenalin) beim Nutztier

Das Ziel der vorliegenden Studie war es, grundlegende Informationen über Metabolisierung und Ausscheidung von Katecholaminen (CA) beim Schaf und Schwein zu bekommen und abzuklären, ob es möglich ist, Enzymimmunoassays für die relevanten Kotmetaboliten zu entwickeln. Dazu wurde der Anteil und zeitliche Verlauf der ausgeschiedenen Radioaktivität in Harn und Kot nach i.v. Verabreichung von <sup>3</sup>H-Adrenalin (<sup>3</sup>H-A) und <sup>3</sup>H-Noradrenalin (<sup>3</sup>H-NA; beide 3,7 MBq) an 4 Schafe (2 pro Geschlecht; insgesamt 8 Applikationen) und 2 Sauen bestimmt. Die CA wurden hauptsächlich über den Harn ausgeschieden (>96 %). Die höchste Radioaktivität im Harn (pro Liter) wurde in einer der 2 unmittelbar nach der Injektion abgesetzten Proben festgestellt. Die Maximalkonzentrationen im Kot wurden hingegen mit einer Verzögerung von 12 h (Schaf) bzw. 48 h (Schwein) gemessen. Danach sank die Radioaktivität langsam ab und erreichte innerhalb von 4 Wochen Hintergrundwerte. Im Kot beider Tierarten waren hauptsächlich Katecholaminmetaboliten vorhanden. Diese Ergebnisse zeigen, dass aufgrund der geringen Mengen an Metaboliten von injizierten Katecholaminen eine quantitative Bestimmung im Kot wahrscheinlich nicht möglich ist.

Abbreviations: A = adrenaline; ACTH = adrenocorticotrophic hormone; CA = catecholamines; EIA = enzyme immunoassay; HPA = hypothalamic-pituitary-adrenocortical axis; HPLC = high performance liquid chromatography; MHPG = 3-methoxy-4-hydroxyphenylglycol; NA = noradrenaline; SA = sympatho-adrenomedullary system; TRR = total recovered radioactivity; VMA = vanillyl-mandelic acid

## Introduction

The front line hormones to overcome stressful situations are the glucocorticoids and catecholamines (CA). The measurement of circulating levels of these hormones has provided information on the effect of acute environmental and psychological challenges in farm animals (DANTZER and MORMEDE, 1983). However, little is known about how long lasting conditions affect neuroen-

docrine activity. Indeed, chronic activation of the sympatho-adrenomedullary system (SA) and hypothalamic-pituitary-adrenocortical (HPA) axis is usually associated with moderate increase in CA and glucocorticoids, respectively. Because of the pulsatility and the circadian variations in the secretion of these hormones, samples must be collected very frequently to obtain reliable basal values. Moreover, the blood sampling procedure may itself induce acute stress responses, thus interfering with the factors under

investigation. Therefore, feedback free sampling methods are preferential (HAY et al., 2000). To overcome these problems, some researchers used special remote blood sampling devices for measuring glucocorticoids (COOK et al., 2000). The technology of measuring heart rate and other physiological parameters with (implantable) transmitters was developed and applied (SCHÖBER et al., 1982), to judge rapid individual adrenergic responsiveness to stress. As heart rate is only an indirect measure of adrenergic responsiveness, several authors have investigated non-invasive sampling procedures, such as a determination of CA metabolites in the urine of humans (for references see NIKOLAJSSEN and HANSEN, 2001), sheep (PAYNE et al., 1992) and sows (LUN et al., 1976; HAY and MORMEDE, 1998; HAY et al., 2000). However, urine collection also involves some manipulation of the animal, is not practicable under everyday conditions and can be used only to a limited extent in free moving animals. Above all, faecal samples offer the advantage that they can be easily collected and this procedure is feedback free. Recently, enzyme immunoassays (EIAs) have been developed and successfully validated to enable the measurement of groups of glucocorticoid metabolites in the faeces of different animal species including sheep (for review see: MÖSTL and PALME, 2002; BINDER et al., 2004; MÖSTL et al., 2005; PALME, 2005; TOUMA and PALME, 2005).

Studies about the fate of synthetic drugs (mainly orally administered) like clenbuterol, which is a  $\beta$ -adrenergic agonist, revealed their predominant urinary excretion in livestock (for review see SMITH, 1998). However, literature concerning the excretion of naturally occurring CA via the faeces is not available. In our study, emphasis was placed on the route of excretion of CA and the time course of the excreted radioactivity in urine and faeces after injecting  $^3\text{H-A}$  and  $^3\text{H-NA}$  in sheep and sows. In addition the main faecal metabolites were characterised. This should clarify, whether or not methods for the determination of some of the relevant faecal metabolites can be developed to allow a non-invasive evaluation of sympathoadrenal activity during disturbances in these animals.

## Material and methods

### Animals

A total of 4 (2 of each sex) mature, healthy sheep and 2 sows from the research farm of the University of Veterinary Medicine, Vienna were used (permission: GZ 68.205/25-Pr/4/2000). Sheep (Mixed breed; 50-60 kg) and sows (German Edelschwein; 200 kg) were 1-2 years old. During the experiment (4-7 days) the animals were kept in a metabolic cage (190 x 60 x 100 cm; length x width x height, respectively) at the Clinic for Ruminants (University of Veterinary Medicine, Vienna). In this specially designed box the animals stand on a tough plastic floor with a pore size of 1 cm<sup>2</sup> to allow the faecal pellets of sheep to pass through and be collected in a metal net beneath this floor. Afterwards the animals were transferred to a stall at the clinic. Sheep were fed with a second cut batch of hay (approximately 1.5-2 kg/day). Sows were fed twice daily with a total of about 2.5 kg per day of a commercially available complete diet based on cereals (supplemented with vitamins and minerals). Animals drank water ad libitum and had access to a lick block of mineral salts. Liver and kidney functions were

determined before the experiment started by measuring activities of diagnostically relevant enzymes, total bilirubin, urea and creatinine in serum. The health status of the animals was checked daily by clinical examination of pulse, body temperature and respiratory rate. All animals remained healthy throughout the experiment.

### Administration of $^3\text{H-CA}$

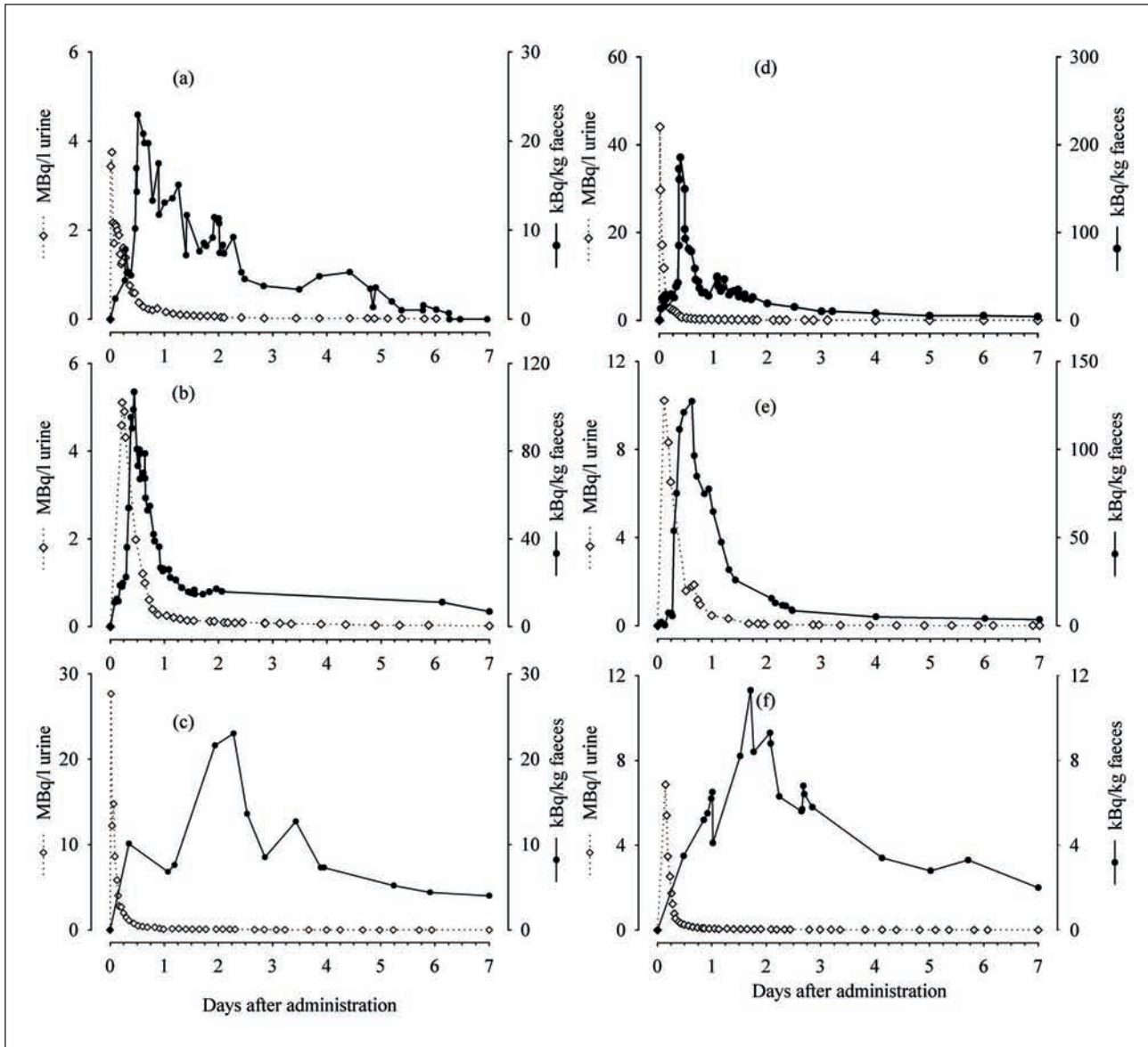
$^3\text{H-A}$  and  $^3\text{H-NA}$  were administered (i.v.) to 2 ewes and 2 rams (total of 8 administrations; EL-BAHR et al., 2005), and two sows.  $^3\text{H-A}$  (special synthesis, WS03DC, epinephrine, levo-ring-2,5,6- $^3\text{H}$ ) and  $^3\text{H-NA}$  (NET 678, norepinephrine, levo-ring-2,5,6- $^3\text{H}$ ) were obtained from New England Nuclear (Boston, USA; purity >97%; specific activity: 40-80 Ci/mmol). The  $^3\text{H-CA}$  (3.7 MBq = 0.1 mCi) were administered in 10 ml sterile 0.9% NaCl solution. The injection lasted for approximately one minute using a canulation (Vasocan-Braunüle®, 0.8 mm x 25 mm, B. Braun, Melsungen, Germany) of the jugular vein in sheep and the ear vein in sows.

### Collection of the samples

The animals were observed continuously for the first 48 h and then at intervals of 2-3 h (with longer intervals of up to 10 h during the night) for the next 2-5 days. In females, urine was collected through inflatable catheters (No. 12 and 14; Rüscher, Kernlen, Germany) to avoid contamination of faeces with urine and to minimise any loss of urine. Urine collections were made at 20-30 minutes intervals until 8 h after the injection, at 60-90 minutes intervals for a further 8 h and then at every 2-3 h until 48 h after the start of the injection. In rams, urine was collected with a detachable collection apparatus (urine funnel) according to PALME et al. (1996). During the first 2 days, urine samples from rams and all faecal material from all animals were taken immediately after voidance. Because CA are stable under acidic conditions, the urine was preserved by adding acetic acid (0.2 mol/l) in the collecting bottle as described by NIKOLAJSSEN and HANSEN (2001). Afterwards, samples were collected at intervals, when animals were observed. Single urine and faecal samples were taken prior to the injection of  $^3\text{H-CA}$  to determine background levels of radioactivity and after 1, 2, 3 and 4 weeks. Urine (n = 456) and faecal samples (n = 477) were immediately frozen and stored at -24 °C. Aliquots of the urine samples for measuring the radioactivity were taken immediately after collection.

### Measurement of radioactivity and calculations

Duplicates of urine samples (0.5 ml) were mixed with 6 ml scintillation fluid (Quicksafe A, No. 1008000, Zinsser Analytic, Maidenhead, UK) and measured in a liquid scintillation counter (Packard Tricarb 4640, Perkin Elmer, Warrenville, IL, USA). A portion of 0.5 g per 50 g of homogenised faeces of each sample was used to determine radioactivity in the faeces. The samples (n = 1,810) were extracted with 0.5 ml distilled water, 0.5 ml acetic acid (0.2 mol) and 4 ml methanol. After shaking (30 min), the mixture was centrifuged at 3,000 g for 15 minutes. Aliquots (0.5 ml) of the supernatant (in duplicate) were measured in the liquid scintillation counter as described previously (TOUMA et al., 2003). Radioactivity was expressed as disintegrations per second (Bq) per kilogram faeces or litre



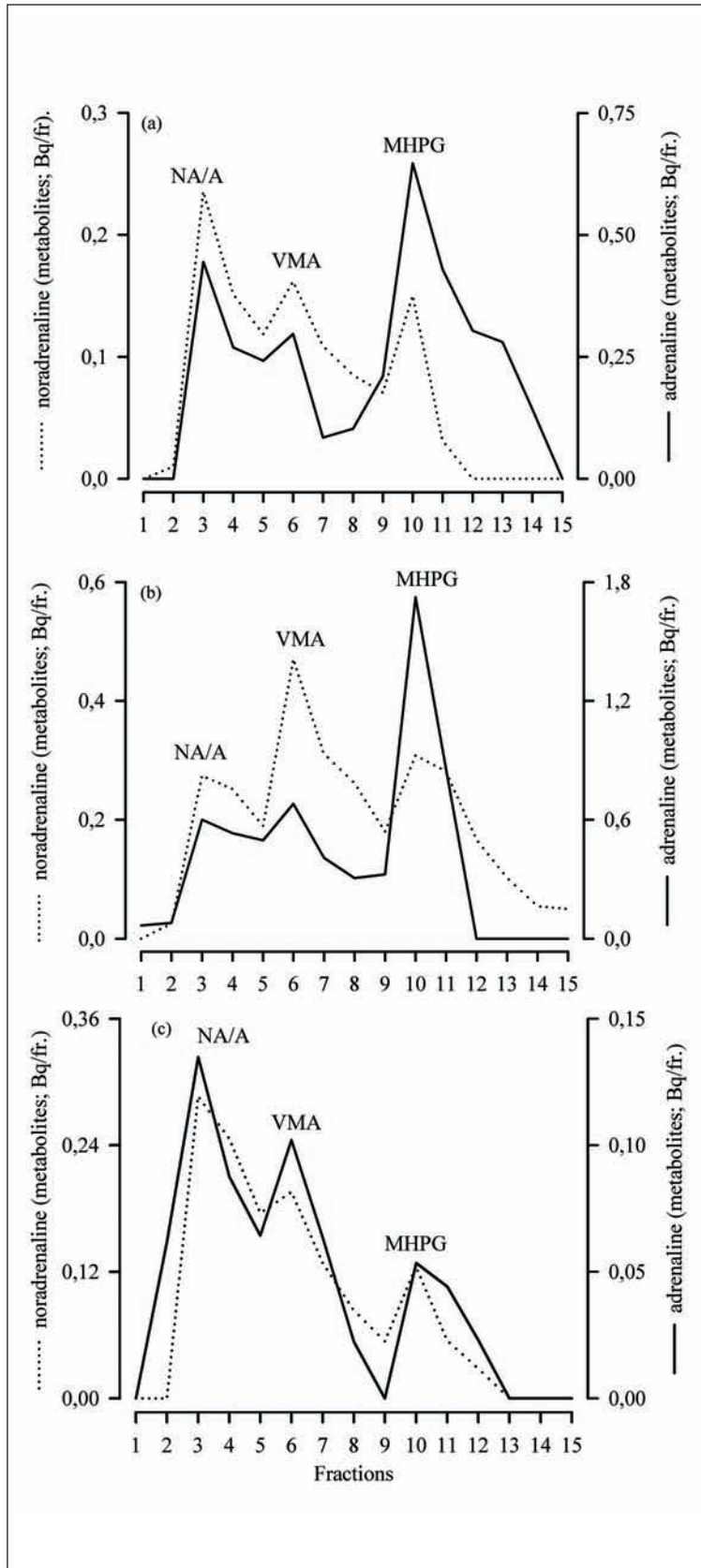
**Fig. 1:** Radioactivity after injection of  $^3\text{H}$ -noradrenaline (a, b and c) and  $^3\text{H}$ -adrenaline (d, e and f) in urine (...◇...) and faeces (—●—) of ewes (a,d), rams (b,e) and sows (c,f), respectively (note the different scale of the y axes)

urine. The injection vial and syringe were rinsed with ethanol after the injections and checked for residual radioactivity. This portion was subtracted from the whole dose to calculate the actual amount of injected  $^3\text{H}$ -CA. The portions of  $^3\text{H}$ -CA metabolites excreted via faeces and urine are expressed as a percentage of the radioactivity recovered. Total recovered radioactivity (TRR; %) was the ratio of the combined sums of the recovered radioactivity in urine and faeces divided by the total amount of administered radioactivity.

#### Characterisation of faecal CA metabolites

To characterise the metabolites, portions of 1 g of homogenised faeces ( $n = 10$ ; samples containing peak radioactivity of each administration of sheep and sows) were used. They were mixed with 50 ml of 1 M HCl. The HCl was used to precipitate the tissue and faecal debris and acted as a bactericide. After shaking for 10 minutes,

the mixture was centrifuged at 3,000 g for 15 minutes. Aliquots (25 ml) of the supernatant were divided into 2 portions (12.5 ml each) and transferred into new tubes. One of these portions was used to determine the amounts of total radioactivity (unmetabolized CA and its metabolites together). The other portion was mixed with 50 ml tris buffer (pH 8.5) and 0.5 g aluminium oxide (alumina; which was acidified by 1 M HCl, then washed three times with distilled water and dried in a sterilization oven immediately before use). After shaking and centrifugation, the supernatant contained the CA metabolites alone. The settled alumina, which forms cyclic complexes with the 3,4-dihydroxyphenyl structure of the CA (KAGEDAL and GOLDSTEIN, 1988), was washed 3 times with distilled water. Subsequently, the water was decanted and the already washed, settled alumina was mixed with 12.5 ml HCl (0.5 M) to extract the unmetabolized CA from the alumina. After shaking and centrifugation, the supernatant contained the



**Fig. 2:** HPLC-separation of  $^3\text{H}$ -noradrenaline (.....) and  $^3\text{H}$ -adrenaline (—) metabolites in the faeces of ewes (a), rams (b) and sows (c); A: adrenaline; NA: noradrenaline; VMA: vanillylmandelic acid; MHPG: 3-methoxy-4-hydroxyphenylglycol

unmetabolized CA. Aliquots (0.5 ml) of all portions were mixed with 6 ml scintillation fluid and the amount of radioactivity was measured in the liquid scintillation counter and values corrected for dilution. The recovery of this procedure was determined to be  $91 \pm 2\%$  (for both CA).

Another portion (1 g) of the same faecal samples was extracted and centrifuged as described above. The whole supernatant was decanted into a new tube and evaporated at  $60^\circ\text{C}$  under a stream of nitrogen. Samples were reconstituted with  $120\ \mu\text{l}$  acetic acid (0.2 M) and then centrifuged. The supernatant ( $100\ \mu\text{l}$ ) of each sample was mixed with  $10\ \mu\text{l}$  of different standards (1 mg/ml acetic acid; A, NA, 3-methoxy-4-hydroxyphenylglycol [MHPG] and vanillylmandelic acid [VMA]; all purchased from Sigma, St. Louis, MO, USA) and separated on a reversed phase column (Nucleosil, 100-C18, G82832;  $250 \times 4\ \text{mm}$ , Forschungszentrum Seibersdorf, Austria), coupled to an ultraviolet detector (wavelength 280 nm). Elution patterns were evaluated with the help of the internal standards. The mobile phase was acetic acid (0.2 mol/l). The flow rate was 1 ml/min and the column was maintained at room temperature. The amount of radioactivity in each fraction (15 fractions of 1 ml per each sample) was measured in the liquid scintillation counter.

Following administration of  $^3\text{H}$ -A and  $^3\text{H}$ -NA the proportion of the radioactivity in the different fractions of an animal was compared with a t-test.

## Results

### Route and time course of excreted radioactivity

An average of  $83 \pm 5\%$  and  $89 \pm 0.7\%$  (mean  $\pm$  SD) of the administered radioactivity was recovered in sheep and sows, respectively. The amounts (%) of the TRR excreted via urine and faeces and the delay times (h) of the maximal concentration of radioactivity after the injection of  $^3\text{H}$ -A and  $^3\text{H}$ -NA in urine and faeces are given in Tab. 1. Administered CA were excreted mainly in the urine ( $>96\%$ ; see Tab. 1 for individual values) and only a small portion ( $2.5 \pm 0.9\%$  and  $1.2 \pm 0.4\%$ ; mean  $\pm$  SD) was found in the faeces of sheep and sows, respectively. Representative graphs of injections of each hormone in each species are given in Fig. 1. In the urine, the peak (4-44 MBq/l) of radioactivity was detected in one of the first 2 samples in all animals. Faecal peak concentrations (23-186 kBq/kg and 11-23 kBq/kg) were measured at about  $12.5 \pm 4.2\ \text{h}$  and  $48 \pm 3\ \text{h}$  after the injection in sheep and sows, respectively. However, in all administrations background levels of radioactivity were reached only after one month. In contrast to the increase of radioactivity, the decline was protracted in the excreta. Especially in females during this phase additional minor

**Tab. 1:** Percentage of total recovered radioactivity (TRR), percentage of excretion via faeces and urine and the delay time (h) of the peak radioactivity after injection of  $^3\text{H}$ -adrenaline (A) and  $^3\text{H}$ -noradrenaline (NA) in sheep (I-VIII) and sows (1-2)

Administration	Sex	Hormone	TRR	Percentage in		Delay time (h)	
				Faeces	Urine	Faeces	Urine
I	f	NA	89.7	2.3	97.7	12.2	0.7
II	f	NA	90.8	1.6	98.4	19.7	1.7
III	f	A	79.3	3.9	96.1	7.3	0.4
IV	f	A	82.7	1.4	98.6	15.7	0.4
V	m	NA	83.7	2.8	97.2	7.8	6.4
VI	m	NA	87.0	3.4	96.7	10.5	5.2
VII	m	A	65.1	2.8	97.2	11.3	5.2
VIII	m	A	84.8	2.1	97.8	15.1	4.9
1	f	NA	88.8	1.4	98.6	45.1	0.3
2	f	A	89.9	0.9	99.1	51.1	0.3

peaks of faecal radioactivity were observed (Fig. 1).

### Characterisation of faecal CA metabolites

Alumina extraction of faecal radioactivity revealed that CA were heavily metabolized. Radioactive substances with a catechol structure represented only a portion of  $12\% \pm 3\%$  in sheep and  $11\%$  in sows, respectively. After liquid chromatographic separation of  $^3\text{H}$ -A and  $^3\text{H}$ -NA metabolites, 3 radioactive peaks, eluting around fractions 3, 6 and 10 were detected (Fig. 2). Their chromatographic property was similar to A/NA, VMA and MHPG, respectively. The elution pattern was the same in sheep and sows. There were no significant differences observed in the metabolism of A and NA ( $t$ -test,  $p = 0.994$ ).

## Discussion

A multitude of hormones such as adrenocorticotrophic hormone (ACTH), glucocorticoids, CA and prolactin is involved in the stress response (MATTERI et al., 2000). The adrenal glands play a key-role in the hormonal stress reactions, as they are involved both in the HPA axis and the SA system (MOBERG, 2000). Adverse situations trigger responses of the adrenals, which result in an increase in glucocorticoid and/or CA secretion. These hormones can be determined in peripheral blood as a parameter of adrenal activity and thus of disturbances. The concentration of glucocorticoids (or their metabolites) can be measured in various body fluids or excreta. Above all, faecal samples offer the advantage that they can be easily collected and this procedure is feedback free (MÖSTL and PALME, 2002). During the last years EIAs have been developed and successfully validated, enabling the measurement of groups of glucocorticoid metabolites in the faeces of different species (for review see: MÖSTL and PALME, 2002; MÖSTL et al., 2005; PALME, 2005; TOUMA and PALME, 2005).

CA are extensively metabolized and quickly (half life of a few minutes) eliminated from the blood (GRANNER, 1996). Determination of their metabolites like VMA in the urine is possible (LUN et al., 1976; PAYNE et al., 1992; HAY et al., 2000). However, literature concerning the excretion of CA via the faeces is not available. In our study, emphasis was placed on the route of excretion of CA and

the time course of the excreted radioactivity in urine and faeces after injecting  $^3\text{H}$ -A and  $^3\text{H}$ -NA in sheep and sows. In addition, the main faecal metabolites were characterised. This should clarify, whether or not methods for the determination of some of the relevant faecal metabolites can be developed to allow a non-invasive evaluation of sympathoadrenal activity during disturbances.

The TRR in our study was comparable to reported experiments with  $^3\text{H}$ -steroids (PALME et al., 1996). In 2 administrations of sheep (III and VII) the TRR of A was lower due to some urine loss during the experiment. The amounts of faecal metabolites of the recovered radioactivity from the injected  $^3\text{H}$ -A and  $^3\text{H}$ -NA were only a few percent, which seem to be insufficient for measuring CA or their metabolites in the faeces. However, the absolute percentage of faecal excretion is not the only criterion, which matters. For example, although comparable small amounts of oestrogens (2%) are excreted in the faeces of mares (PALME et al., 1996), a pregnancy diagnosis based on their determination had been successfully established (MÖSTL et al., 1983; PALME et al., 1989). The other alternative sample material, which can be collected non-invasively, is the urine, as it contained almost all radioactivity (>96%) excreted. Although CA and/or their metabolites have been measured in the urine of domestic livestock (LUN et al., 1976; PAYNE et al., 1992; HAY and MORMEDE, 1998; HAY et al., 2000), sampling itself has the disadvantages of being less practical and/or disturbing the animal.

Means ( $\pm$  SD) of the radioactivity in samples of all injections of one hormone were not calculated, since the maximal concentrations and the time course of excretion depended upon the intervals between and the amounts of voided samples. Therefore differences in the concentrations of radioactivity in the excreta were found between the administrations. In rams, urine was sampled after spontaneous voidance and consequently the time of maximal radioactivity in urine could not be defined as exactly as in ewes and sows. The urinary samples did not reflect the radioactivity of the urine actually produced but only the pooled concentration of  $^3\text{H}$ -CA metabolites for the whole period since the last urination related to the time of voidance. The predominant excretion of A and NA via the urine resulted in a quick elimination of the main portion of administered radioactivity. However, contrary to steroids (PALME

et al., 1996), in all administrations background levels of radioactivity in the excreta were reached only after 1 month. The long lasting radioactivity in urine and faeces of sheep and sows might be due to the binding of CA and/or their metabolites to plasma proteins as was shown in (in vitro) experiments (MAY et al., 1974; SAGER et al., 1987; EL-BAHR et al., 2005). This was underlined by the fact that a similar phenomenon in the plasma samples of those sheep was found (EL-BAHR et al., 2005). The delay times of faecal CA excretion (about 12 h in sheep and 48 h in sows) were the same, as those reported for steroids, indicating a similar excretion (from the liver via the bile into the gut; PALME et al., 1996). The occurrence of additional peaks of radioactivity after the maximum in the faeces may be due to an enterohepatic circulation as was found in steroids (PALME et al., 1996), which is more likely to be seen in ewes as they defecated more often.

As many studies (e.g.: LUN et al., 1976; PAYNE et al., 1992) have described urinary CA and/or their metabolites in sheep and swine, our study concentrated on the characterization of faecal CA. Alumina was used for selective purification of faecal radioactivity, as it forms cyclic complexes with the 3,4-dihydroxyphenyl structure of the CA (KAGEDAL and GOLDSTEIN, 1988). In the faeces substances with such a catechol structure accounted for only about 12 and 11 % in sheep and sows, respectively. The rest represented metabolites. As reported in sheep (PAYNE et al., 1992) and sows (LUN et al., 1976) for the urine, VMA and HMPG were also main metabolites in the faeces. Because CA are extensively metabolized, different metabolites might elute in the same peak. As only low concentrations of radioactivity were present in the faeces, it was not possible to use more sophisticated HPLC systems or to collect more fractions, as the low radioactivity could not be discriminated from the background levels. This was the reason why the CA metabolites could only be characterised and not identified. The low amounts of faecal CA metabolites seem to be insufficient for quantitative measurement, especially as the concentration of plasma CA is in the picogram range, increasing the problem for measuring it in faeces. In addition, the secretion of CA from hepatomesentric organs should be taken into consideration (EISENHOFER et al., 2001). However, antibodies against VMA (the main metabolite of A and NA) should be produced to establish an EIA for the determination of a relevant faecal CA metabolite, thereby perhaps allowing a non-invasive evaluation of disturbances in other species like birds, where urine and faeces are excreted together in the form of droppings.

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### References

BINDER, R., PALME, R., SOMMERFELD-STUR, I., BAUMGARTNER, J., NIEBUHR, K., ZALUDIK, K., BARTUSSEK, K., WAIBLINGER, S., REIFINGER, M., KÜBBER-HEISS, A., RABITSCH, A., GNEIST, M., TROXLER, J. (2004): Tierschutz in Österreich: Grundkonzepte der Tierethik, rechtliche Rahmenbedingungen

und ausgewählte Schwerpunkte der Tierschutzforschung. *Vet. Med. Austria/ Wien. Tierärztl. Mschr.* **91** (Suppl. 1), 44-58.

COOK, C.J., MELLOR, D.J., HARRIS, P.J., INGRAM, J.R., MATTHEWS, L.R. (2000): Hand-on and hand-off measurement of stress. In: MOBERG, G.P., MENCH, J.A. (eds.): *The biology of animal stress*. CABI Publishing, Oxon, New York, p. 123-146.

DANTZER, R., MORMEDE, P. (1983): Stress in farm animals: a need for reevaluation. *J. Anim. Sci.* **57**, 6-18.

EISENHOFER, G., HUYNH, T.T., HIROI, M., PACAK, K. (2001): Understanding catecholamine metabolism as a guide to the biochemical diagnosis of pheochromocytoma. *Endocr. Metab. Disorders* **2**, 297-311.

EL-BAHR, SM., KAHLBACHER, H., PATZL, M., PALME, R.G. (2005): Clearance and binding systems of radiolabelled adrenaline and noradrenaline in sheep blood. *Vet. Res. Commun.* (in press).

GRANNER, D.K. (1996): Hormones of the adrenal medulla. In: MURRAY, R.K., GRANNER, D.L., MAYES, P.A., RODWELL, V.W. (eds.): *Harper's Biochemistry*. Prentice-Hall International, Inc., London, p. 560-565.

HAY, M., MORMEDE, P. (1998): Urinary excretion of catecholamines, cortisol and their metabolites in meishan and large white sows: validation as a non-invasive and integrative assessment of adrenocortical and sympathoadrenal axis activity. *Vet. Res.* **29**, 119-128.

HAY, M., MEUNIER-SALAÜN, M.C., BRULAUD, F., MONNIER, M., MORMEDE, P. (2000): Assessment of hypothalamic-pituitary-adrenal and sympathetic nervous system activity in pregnant sows through the measurement of glucocorticoids and catecholamines in urine. *J. Anim. Sci.* **78**, 420-428.

KAGEDAL, B., GOLDSTEIN, D.S. (1988): Catecholamines and their metabolites. *J. Chrom.* **429**, 177-233.

LUN, A., HACKER, R.R., BROWN, R.G., HURNIK, J.F. (1976): Determination of vanilmandelic acid in pig urine and chicken faeces by gas liquid chromatography. *Anal. Biochem.* **73**, 267-273.

MATTERI, R.L., CARROL, J.A., DYER, C.J. (2000): Neuroendocrine responses to stress. In: MOBERG, G.P., MENCH, J.A. (eds.): *The biology of animal stress*. CABI Publishing, Oxon, New York, p. 43-76.

MAY, P.H., SANDERS, F.J., DONABEDIAN, R.K. (1974): Binding of catechol derivatives to human serum proteins. *Experientia* **30**, 304-305.

MOBERG, G.P. (2000): Biological response to stress: implication for animal welfare. In: MOBERG, G.P., MENCH, J.A. (eds.): *The biology of animal stress*. CABI Publishing, Oxon, New York, p. 123-146.

MÖSTL, E., PALME, R. (2002): Hormones as indicators of stress. *Dom. Anim. Endocrinol.* **23**, 67-74.

MÖSTL, E., NÖBAUER, H., CHOI, H.S., WURM, W., BAMBERG, E. (1983): Trächtigkeitsdiagnose bei der Stute mittels Östrogenbestimmung im Kot. *Prakt. Tierarzt* **64**, 491-492.

MÖSTL, E., RETTENBACHER, S., PALME, R. (2005): Measurement of corticosterone metabolites in birds' droppings: an analytical approach. *Ann. N. Y. Acad. Sci.* **1046**, 17-34.

NIKOLAJSEN, R.P.H., HANSEN, A.M. (2001): Analytical methods for determining urinary catecholamines in healthy subjects. *Anal. Chim. Acta* **449**, 1-15.

PALME, R. (2005): Measuring fecal steroids: guidelines for practical application. *Ann. N. Y. Acad. Sci.* **1046**, 75-80.

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

PALME, R., MÖSTL, E., BAMBERG, E., LORIN, D., ARBEITER, K. (1989): Sicherheit der Trächtigkeitsbestimmung bei der Stute mittels Östrogenbestimmung im Kot. *Prakt. Tierarzt* **69**, 43-44.

PAYNE, E., COPE, B.C., HUGHES, J.M., PHIPPS, D.E. (1992): The metabolism of noradrenaline in the sheep and the effect of dry matter intake upon the production of a metabolite urinary vanilmandelic acid. *Comp. Biochem. Physiol. C* **101**, 661-664.

- SAGER, G., BRATLID, H., LITTLE, C. (1987): Binding of catecholamines to alpha-1 acid glycoprotein, albumin and lipoproteins in human serum. *Biochem. Pharm.* **36**, 3607-3612.
- SCHOBER, F., PASTERK, W., SOMMER, F. (1982): Implantable long-range, long-life, heart rate and activity transmitter for free-ranging animals. In: MEINDL, J.D., KNUTTI, J.W. (eds.): *Biotelemetry VII. Proc. 7<sup>th</sup> Int. Symp. Biotelemetry, Stanford, CA*, p. 108-111.
- SMITH, D.J. (1998): The pharmacokinetics, metabolism, and tissue residues of  $\beta$ -adrenergic agonists in livestock. *J. Anim. Sci.* **76**, 173-194.
- TOUMA, C., PALME, R. (2005): Measuring fecal glucocorticoid metabolites in mammals and birds: the importance of a validation. *Ann. N. Y. Acad. Sci.* **1046**, 54-74.
- TOUMA, C., MÖSTL, E., SACHSER, N., PALME, R. (2003): Effect of sex and time of day on metabolism and excretion of corticosterone in urine and feces of mice. *Gen. Comp. Endocrinol.* **130**, 267-278.

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**Atlas der Anatomie des Pferdes. Lehrbuch für Tierärzte und Studierende.** Von K.-D. BUDRAS und S. RÖCK. 5., vollst. überarb. Aufl. Schlütersche Verlagsgesellschaft, Hannover, 2004. 192 S., ca. 200, teils großformatige Abb., EUR 76,-, ISBN 3-89993-002-9.

Der „Atlas der Anatomie des Pferdes“ setzt auch in der aktuellen, vollständig überarbeiteten Auflage die bewährte Konzeption dieser Anatomieatlanten als Standardwerk für Studium und Praxis fort. Insbesondere das bewährte didaktische Konzept einer Gegenüberstellung von deskriptiven und illustrativen Elementen auf gegenüberliegenden Buchseiten ermöglicht eine integrative Wissensvermittlung. Die Topographische Anatomie ist in 9 Kapitel unterteilt: Haut, Schultergliedmaße, Beckengliedmaße, Kopf, Zentralnervensystem, Stammskelett und Hals, Brusthöhle, Bauchwand und Bauchhöhle, Becken mit Leistenengegend sowie Harn- und Geschlechtsorgane. Die Abbildungen sind von höchster Qualität, detailliert beschriftet, ohne beim Betrachten unübersichtlich zu wirken und fördern ein besseres Verständnis der anatomischen Zusammenhänge. Zusätzlich findet sich eine umfangreiche Beschreibung ausgewählter Themengebiete in einem weiteren Kapitel „Anhang spezielle Anatomie“. In der Myologie wird jeder Muskel mit Namen, Ursprung, Ansatz, Innervation, Funktion und besonderen Anmerkungen, Ausnahmen und Faserverläufen beschrieben. In der Lymphologie sind alle Lymphzentren angeführt, unter besonderer Berücksichtigung der zugehörigen Lymphknoten, ihrer Lage, dem tributären Gebiet und dem Abfluss. Weiterführend finden sich besondere Anmerkungen hinsichtlich ihrer Topographie, Form, Anzahl und Regelmäßigkeit des Auftretens sowie Bedeutung im Rahmen der Fleischschau. Beim peripheren Nervensystem wird das Innervationsgebiet der Nerven angegeben und überblickshaft deren Verlauf beschrieben. Bei den Gehirnnerven werden deren Aufteilungen und Faserqualitäten, sowie deren Innervationsgebiete ausführlich dargestellt.

Zur Veranschaulichung der Muskeln, Lymphzentren und Nerven ist (sind) konsequent die zugehörige(n) Abbildung(en) im topographischen Teil des Atlas angegeben, wodurch ein Auffinden im Werk wesentlich erleichtert ist. Ca. 60 Seiten des Werkes sind insbesondere Beiträgen zur klinisch-funktionellen Anatomie gewidmet. In diesem Teil findet der Leser Informationen zur Arthroskopie und Endoskopie, zu häufigen organspezifischen Erkrankungen und Abbildungen zu deren Diagnostik unter Verwendung von Röntgenaufnahmen oder Ultraschallbildern. Weiters sind Punktionsstellen von Gelenken und Injektionsstellen zur Leitungsanästhesie in Text und Bild verdeutlicht, wobei als Ergänzung Querschnitte von Extremitäten abgebildet sind. Zusammenfassend kann dieses Werk sowohl als Grundlage für Studierende als auch als ausgezeichnetes Hilfsmittel für praktizierende Tierärzte empfohlen werden. Vorteilhaft ist, dass umfangreiches und wichtiges anatomisches Fachwissen schnell und übersichtlich verwirklicht wird.

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