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Omer-Elfaroug Sid-Ahmed, Ahmed Sanhouri, Badr-Eldin Elwaseela, Imad Fadllalah, Galal-Eldin Elazhari Mohammed & Erich Möstl

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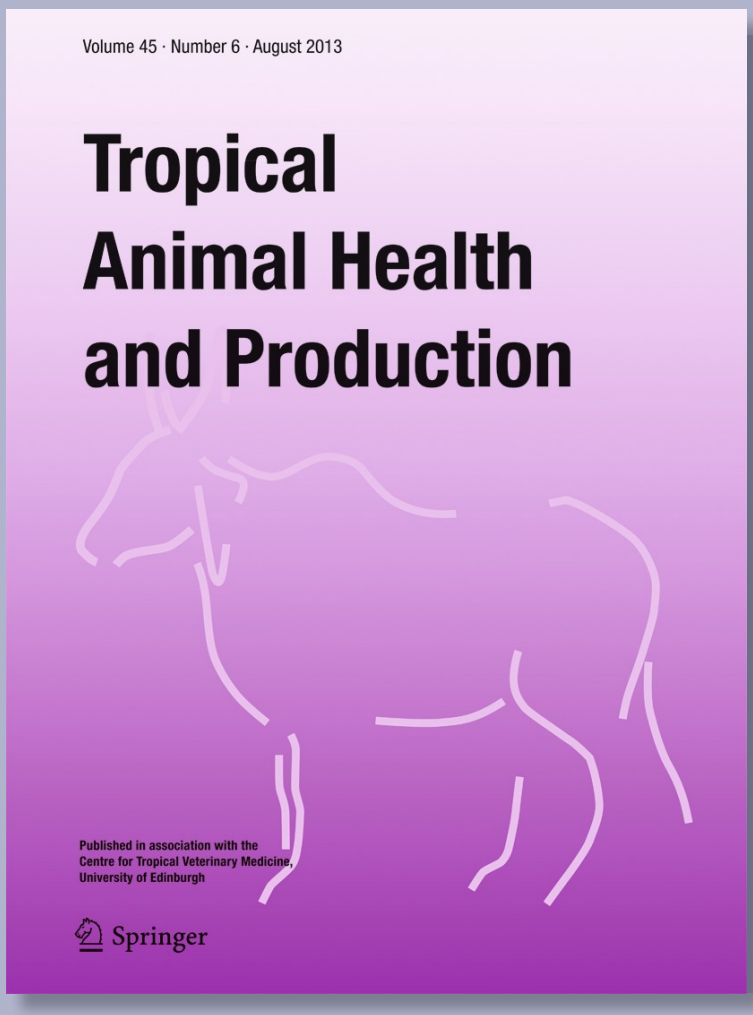

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SHORT COMMUNICATIONS

Assessment of adrenocortical activity by non-invasive measurement of faecal cortisol metabolites in dromedary camels (*Camelus dromedarius*)

Omer-Elfaroug Sid-Ahmed · Ahmed Sanhouri · Badr-Eldin Elwaseela · Imad Fadllalah · Galal-Eldin Elazhari Mohammed · Erich Möstl

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Abstract The aim of this study was to determine whether glucocorticoid production could be monitored non-invasively in dromedary camels by measuring faecal cortisol metabolites (FCMs). Five Sudanese dromedaries, two males and three females, were injected with a synthetic adrenocorticotropic hormone (ACTH) analogue. Blood samples were collected pre- and post-ACTH injection. Faeces were sampled after spontaneous defecation for five consecutive days (2 days before and 3 days after ACTH injection). Baseline plasma cortisol values ranged from 0.6 to 10.8 ng/ml in males and from 1.1 to 16.6 ng/ml in females, while peak values after ACTH injection were 10.9–41.9 in males and 10–42.2 ng/ml in females. Peak blood cortisol values were reached between 1.5 and 2.0 h after ACTH injection. The concentration of FCMs increased after ACTH injection in the faeces of both sexes, although steroid levels peaked earlier in males [24 h; (286.7–2,559.7 ng/g faeces)] than in females [36–48 h; (1,182.6–5,169.1 ng/g faeces)], reflecting increases of 3.1–8.3- and 4.3–8-fold above baseline levels. To detect chromatographic patterns of immunoreactive FCMs, faecal samples with high FCM concentrations from both sexes were pooled and subjected to reverse phase high performance liquid chromatography (RP-HPLC). RP-HPLC analysis revealed sex differences in the polarity of FCMs, with females showing more polar FCMs than males. We concluded that stimulation of adrenocortical activity by ACTH injection resulted in a measurable increase in blood cortisol that was reliably paralleled by increases in FCM levels. Thus, measurement of FCMs is a powerful tool for monitoring the adrenocortical responses of dromedaries to stressors in field conditions.

Keywords Glucocorticoids · Cortisol · ACTH · Stress · Dromedary · Faecal metabolites

O.-E. Sid-Ahmed (✉) · A. Sanhouri · I. Fadllalah · G.-E. E. Mohammed
Department of Biomedical Sciences, College of Veterinary Medicine, Sudan University of Science and Technology, P.O. Box 204, Khartoum, Sudan
e-mail: elfaroug@sustech.edu

B.-E. Elwaseela
Department of Camel Production, Tumbool Camel Research Centre, Animal Resources Research Corporation, P.O. Box 8067, Khartoum, Sudan

O.-E. Sid-Ahmed · E. Möstl
Department of Biomedical Sciences, Institute of Medical Biochemistry, University of Veterinary Medicine, Veterinärplatz 1, 1210 Vienna, Austria

Introduction

Among domesticated animal species, camels are very versatile. Most notably, they are uniquely adapted to withstand harsh and stressful conditions in arid and semi-arid areas where food and water are frequently scarce. Camels have developed physiological and biochemical adaptive patterns to cope and reproduce in harsh grazing conditions (reviewed by Saeb et al. 2010). However, camel production may decrease (Kataria and Kataria 2010) when stress responses are activated too much or for too long, which can lead to impaired reproduction, immunity and growth (reviewed by Palme 2012).

Over the last few decades, camels have been increasingly recognised for their food production potential. However, nutrition and the conditions under which they are produced, transported and slaughtered can influence carcass characteristics

Table 1 Blood cortisol values (ng/ml) before (basal) and after (peak) ACTH challenge in individual male dromedaries

Animal No.	Basal (ng/ml blood)			Peak (ng/ml blood)				Peak time after treatment (h)
	Min	Max	Mean±SEM	Min	Max	Mean±SEM	Increase (fold) after ACTH challenge	
1	0.6	4.8	2.5±1	10.9	41.9	26.4±2.2	16.4	1.75
2	0.9	10.8	4.1±1.5	11	24.9	17.6±1	6.1	1.50

ACTH adrenocorticotropic hormone, *Min* minimum, *Max* maximum, *SEM* standard error of the mean

and other physiological indices (Mohammed et al. 2010). Because of the close relationship between serum levels of the major stress biomarker, cortisol and faecal cortisol metabolites (FCMs), most researchers measure FCMs as a marker of cortisol, as no restraint or casting of the animal is necessary and therefore there are no confounding influences.

The metabolites formed and the percentages of metabolites that are excreted in faeces vary among species (Palme and Möstl 1997; Möstl et al. 1999). Consequently, group-specific enzyme immunoassays (EIAs) that measure FCMs with different chemical configurations have been developed, validated and adopted for use in several domestic animal species as a measure of animal welfare and to assess adrenocortical activity in response to stressful situations (Palme and Möstl 1997; Möstl et al. 1999; Rettenbacher et al. 2004). One EIA that is often used to determine FCMs is the 11-oxoetiocholanolone EIA. This EIA, which was first described by Möstl et al. (2002), measures FCMs with a 3α-ol, 11-one configuration; 11-oxoetiocholanolone is used as a standard. This assay has been used to monitor stress induced by handling and veterinary manipulation of cattle and horses and thus shows promise for the assessment of adrenocortical activity in dromedary camels as well. However, prior to use of any established EIA, it must be validated in the particular animal species (Touma and Palme 2005). The objective of this study was to validate the use of this non-invasive EIA method for monitoring FCMs in dromedary camels.

Materials and methods

Five Sudanese dromedary camels (Western ecotype), two males (1.5–3 years, 230–420 kg BW) and three females (3–12 years, 440–550 kg BW), were selected from the Tumbool Camel Research Centre farm. Females were housed together in metallic enclosures of about 1,600 m^2, while each bull was housed alone in a ~16 m^2 stall. All animals appeared to be healthy. The animals were provided with a concentrated food source (cotton nut cake mixed with molasses) every morning and had access to salt blocks and hay and fresh water ad libitum. Two days prior to blood sampling, a 16 G indwelling permanent catheter was inserted into the jugular vein to avoid any stress induced by catheter insertion. All animals were injected intravenously with adrenocorticotropic hormone (ACTH; 0.5 mg/animal, Synacthen®, Novartis, Basel, Switzerland) into the vena jugularis via the catheter, and blood samples were collected before ACTH injection to assess basal cortisol levels at time intervals of −26.75, −23.25, −10.25, −1.25, −1, −0.75 and −0.5 h relative to the injection time. To measure any increases in cortisol values above basal levels after ACTH injection, samples were collected 0, 0.25, 0.5, 1, 1.25, 1.5, 1.75, 2, 2.5, 3, 3.5, 4, 4.5, 35.25, 48.25, 58.25, 72.25 and 81.25 h post-injection. Faecal samples (two to three balls) were collected after spontaneous defecation between 48 h before till 72 h after ACTH administration. The samples were placed immediately in plastic bags, hand squeezed and kept at −20 °C until extracted. Blood samples were extracted and assayed for cortisol using the method described by Palme and Möstl (1997). Faecal samples were extracted using 80 % methanol according to Palme and Möstl (1997). FCMs were quantified using the EIA method described by Möstl et al. (2002).

The intra- and inter assay coefficients of variation were (5–6.2 %) and (7.9–8.4 %) for serum cortisol, respectively. For FCMs, the intra- and inter assay coefficients of variation were 5.6–7.5 and 8.2–9.4 %, respectively.

Table 2 Blood cortisol values (ng/ml) before (basal) and after (peak) ACTH challenge in individual female dromedaries

Animal No.	Basal (ng/ml blood)			Peak (ng/ml blood)				Peak time after treatment (h)
	Min	Max	Mean±SEM	Min	Max	Mean±SEM	Increase (fold) after ACTH challenge	
1	1.1	9.2	3.4±1.4	16.8	40.2	24.9±1.4	11.7	2.00
2	2	16.4	7±1.8	23.9	42.2	33.7±1.2	6.1	2.00
3	1.9	16.6	7±1.9	10	32.5	19.7±1.4	4.7	1.75

ACTH adrenocorticotropic hormone, *Min* minimum, *Max* maximum, *SEM* standard error of the mean

Table 3 FCMs values (ng/g faeces) before (basal) and after (peak) ACTH challenge in individual male dromedaries

Animal No.	Basal (ng/g faeces)			Peak (ng/g faeces)				Peak time after treatment (h)
	Min	Max	Mean±SEM	Min	Max	Mean±SEM	Increase (fold) after ACTH challenge	
1	164.8	397.3	278.8±4.6	505.2	864.3	650.6±5	3.1	24
2	210.9	489.5	307.6±4.4	286.7	2,559.7	1,687.8±16.2	8.3	24

FCMs faecal cortisol metabolites, *ACTH* adrenocorticotropic hormone, *Min* minimum, *Max* maximum, *SEM* standard error of the mean

To detect the chromatographic patterns of immunoreactive FCMs, faecal samples containing high concentration of FCMs from males and females were pooled separately and subjected to reverse phase high performance liquid chromatography (RP-HPLC). Chromatographic separation was performed according to Möstl et al. (2002). Immunoreactive substances were identified in each RP-HPLC fraction using the cortisol and FCM EIAs.

Individual basal levels (before ACTH) and increased levels (after ACTH) of plasma cortisol and FCMs were expressed as a range for each animal. Due to differences in spontaneous defecation time and defecation frequency, FCMs were grouped in 12-h time intervals before and after ACTH injection. Statistical analysis was performed using SigmaStat® 3.11 (SPSS Inc., Berlin, Germany).

Results

Blood cortisol values before and after ACTH injection are shown for males and females in Tables 1 and 2, respectively. FCM values before and after ACTH injection are shown for males and females in Tables 3 and 4, respectively. Administration of ACTH resulted in an immediate increase in blood cortisol levels in both sexes within a range of 1.50–2.00 h (Fig. 1). Administration of ACTH increased FCM values in a variable manner in both sexes. Maximal peak values were reached 24 h after ACTH injections in males and 36–48 h after ACTH injection in females; FCM levels started to decline after 36 h in males and 48 h in females (Fig. 1).

Figure 1 shows blood cortisol and FCMs values over time in a representative male and female. Stimulation of adrenocortical activity by ACTH injection resulted in a measurable increase in blood cortisol, and this increase was reliably detected in parallel with increases in FCMs. However, the lag time (intestinal passage time) between peak values of blood cortisol and its FCMs differed between males (22 h) and females (34–46 h).

Figure 2 shows immunoreactive cortisol and FCM levels over time in pooled faecal samples from males and females. The main purpose of this analysis was to assess the validity of the FCM assay; specifically, we set out to determine whether cortisol was present in the faecal matrix and to detect chromatographic patterns of FCMs in both male and female camels. Only minor amounts of immunoreactive cortisol were detected in the faecal samples, whereas two major peaks of immunoreactivity were detected in faeces using the FCM EIA. These peaks were eluted between fractions 35–40 and 42–46 (males) and fractions 34–37 and 39–42 (females) and had a polarity like that of unconjugated steroids. Chromatographic separation indicates sex differences in the polarity of excreted faecal metabolites in that females excreted more polar metabolites than males with higher immunoreactivity values in the RP-HPLC fractions as revealed by FCM EIA.

Discussion

Clearly, there are differences in glucocorticoid metabolism and in the excretion of glucocorticoid metabolites within and between species. Thus, it is essential to perform careful measurements to validate a method for use in a particular animal species. The aim of this study was to validate the reliability of measuring FCMs as a reflection of adrenocortical function in dromedaries as a useful non-invasive

Table 4 FCM values (ng/g faeces) before (basal) and after (peak) ACTH challenge in individual female dromedaries

Animal No.	Basal (ng/g faeces)			Peak (ng/g faeces)				Peak time after treatment (h)
	Min	Max	Mean±SEM	Min	Max	Mean±SEM	Increase (fold) after ACTH challenge	
1	365.6	657.7	480.9±3.6	1,465.8	2,628	2,039.6±8.25	5.5	48
2	711.4	2,065.1	1,201.8±9.0	3,982.1	5,169.1	4,406.82±5.9	4.3	36
3	110	518.2	250.4±7.1	1,182.6	2,007.1	1,519.82±6.2	8.1	48

FCMs faecal cortisol metabolites, *ACTH* adrenocorticotropic hormone, *Min* minimum, *Max* maximum, *SEM* standard error of the mean

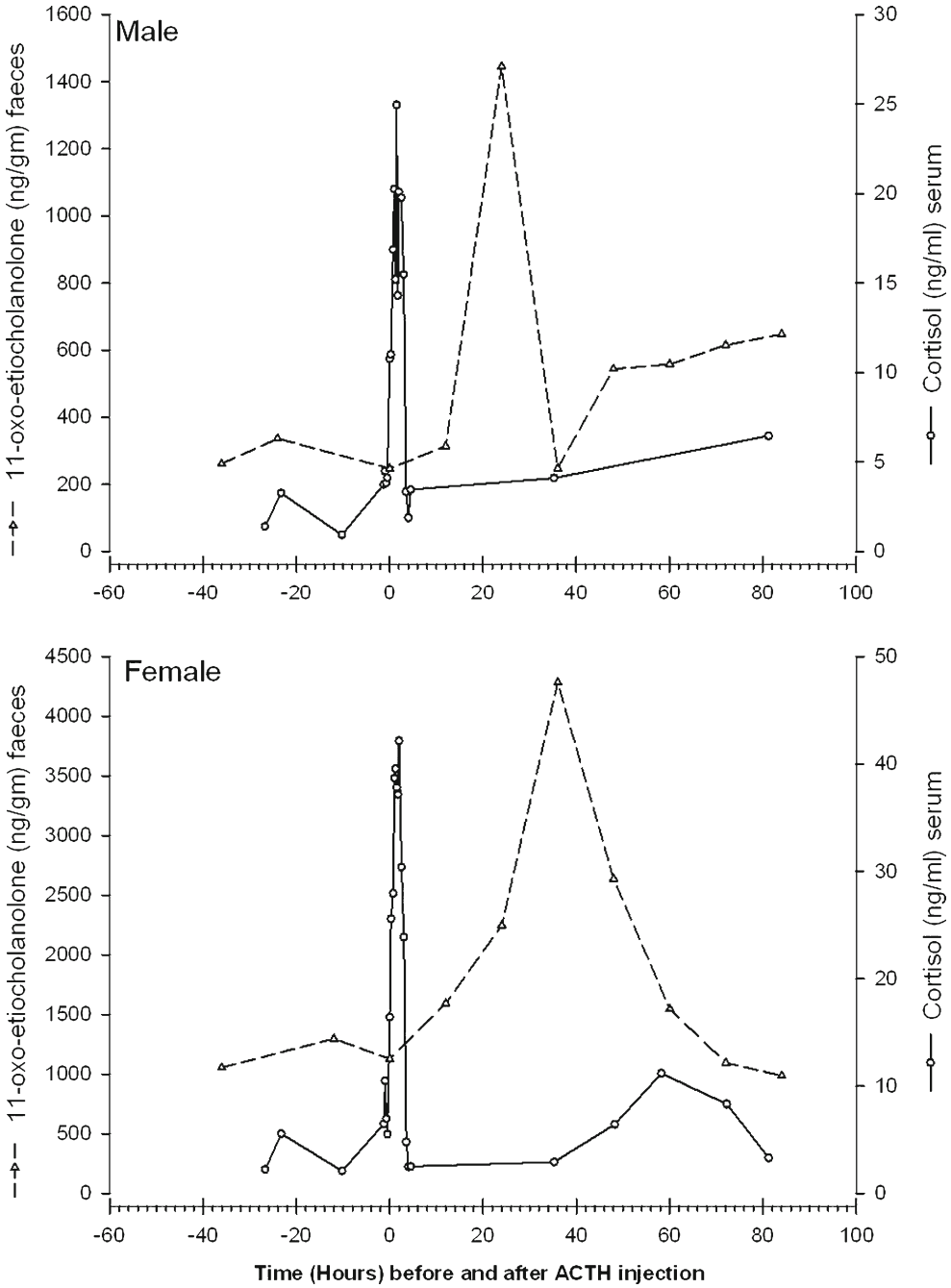

Fig. 1 Serum cortisol and faecal cortisol metabolite (FCM) (11-oxo-etiocholanolone) levels in a representative male dromedary (*upper panel*) and in a representative female dromedary (*lower panel*) before and after adrenocorticotropic hormone (*ACTH*) injection. Note the differences in the time lags for FCMs between the peak levels in both males and females after ACTH injection

technique. Basal values (before ACTH injection) of blood cortisol in this study were lower than those reported in dromedaries by Mohamed (2006) and Saeb et al. (2010). A possible explanation for these differences may be that the two EIAs used to quantify blood cortisol in those studies had different specificities than the EIA we used. To our knowledge, the present study is the first to stimulate adrenocortical activity using ACTH in dromedaries. Others have shown that ACTH injection should result in a significant increase in plasma glucocorticoid values, and this increase should be reflected in the FCM values (Touma and Palme 2005). Pharmacological stimulation of the adrenal gland in dromedaries by injecting ACTH resulted in 4.7–11.7-fold (females) and 6.1–16.4-fold (males) increases in blood cortisol values. In both sexes, peak blood cortisol values were reached 1.50–2.00 h after ACTH injection and were comparable to those reported by Agarwal et al. (1992).

FCMs peaked in all of the animals following injection of exogenous ACTH; however, peak FCM levels were reached earlier in males (24 h) than in females (36–48 h) (Table 2), indicating sex differences in the excretory profiles of FCMs after ACTH injection. It is important to take these results into account in studies investigating FCMs in dromedaries. For example, in laboratory mice, Touma et al. (2003)

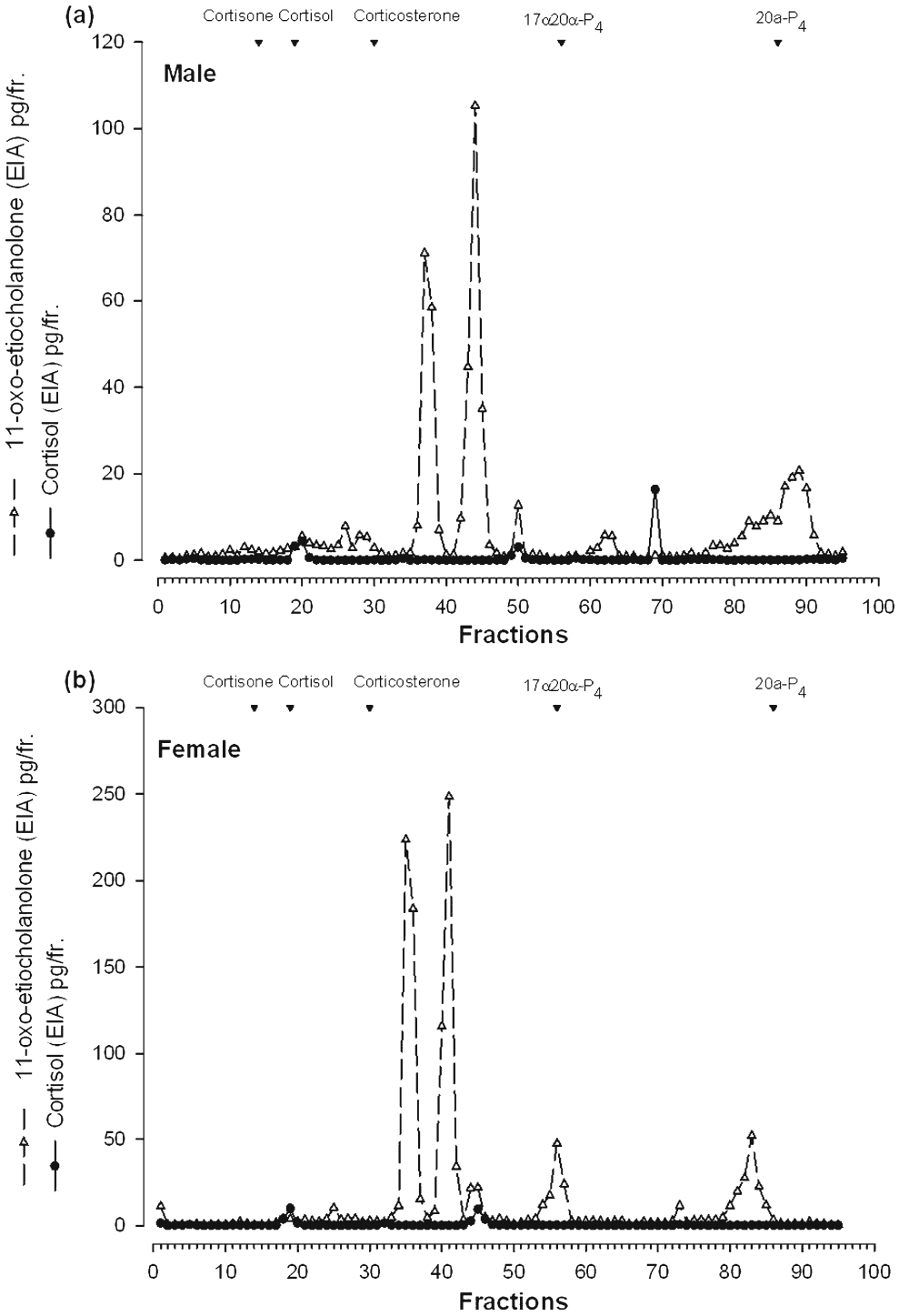

Fig. 2 RP-HPLC profiles of 11-oxo-etiocholanolone and cortisol immunoreactive substances in pooled faeces samples from male (**a**) and female (**b**) dromedaries obtained after adrenocorticotropic hormone (ACTH) injection. The elution positions of the standard steroids are indicated with *black inverted triangles* (cortisone, cortisol, corticosterone, (17α20α): 17α20α-dihydroxyprogesterone, (20αP4): 20α-hydroxyprogesterone). Note the difference in the faecal cortisol metabolite (FCM) polarity in samples from males and females

reported two peaks of corticosterone metabolites, at 4 and 10 h, in faeces post-ACTH injection; this suggested an effect of the time of the day on the peaks of FCMs.

FCM levels paralleled blood cortisol values with a delay time of 22 h (males) and 34–36 h (females; Fig. 1). As glucocorticoid metabolites are eliminated via the bile into the gut, this delay in the appearance of FCMs in the faeces corresponds mainly to the animal intestinal transit (lag) time from the duodenum to rectum (Palme et al. 1996). Different animal species have different gut passage times until FCMS can be detected in the faeces: domestic sheep, 12 h; ponies, 24 h; pigs, 48 h (Palme et al. 1996); African elephants, 48 h (Ganswindt et al. 2003); domestic chickens, 4.7 h (Rettenbacher et al. 2004); and Roe deer, 11.8 h (Dehnhard et al. 2001).

Because the antibody used to measure FCMs was raised against 11-oxoetiocholanolone that was linked at position 17 of the molecule, the assay cross-reacts with 5β-androstanes and 5β-preganes with a 3-α hydroxyl plus an

11-oxo group (Möstl et al. 2002). Therefore, we cannot be sure whether the metabolites that were measured had 19 or 21 C-atoms.

RP-HPLC analysis revealed substantial sex-specific variations in terms of the polarity and the quantity of the FCMs. Females showed higher FCM levels and more polar metabolites compared to males; this is consistent with previous investigations in other animal species that also found that females had more polar faecal metabolites (Touma et al. 2003; Rettenbacher et al. 2004). Notably, inter-female variations were seen in this study (Table 2), and these variations were most likely due to the broad age range (3–12 years) of the selected animals. It is also possible that differences in the distribution of FCMs in faeces could also underlie the inter-individual variations we observed. Cortisol was virtually absent in the faecal matrix (Fig. 2), so it can be assumed that nearly all cortisol was metabolised in the dromedaries, indicating robust steroid metabolism in the liver.

In conclusion, the results of this study showed that increases in serum cortisol values after ACTH injection were paralleled by increases in FCM values, suggesting that measurement of FCMs is a reliable way to monitor adrenocortical activity in dromedaries. This study also provides valuable information about sex-specific differences in the timing of peak levels of FCMs after ACTH injection. Use of this non-invasive technique avoids sampling-induced stress and is an easy way to investigate adaptive endocrine mechanisms in dromedary camels.

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References

Agarwal, S.P., Rai, A.K., and Khanna, N.D., 1992. Hormonal studies in postpartum female camels and their neonates, Theriogenology, 38, 735–747

Dehnhard, M., Clauss, M., Lechner-Doll, M., Meyer, H.H.D., and Palme, R., 2001. Noninvasive monitoring of adrenocortical activity in Roe Deer (*Capreolus capreolus*) by measurement of fecal cortisol metabolites. General and Comparative Endocrinology, 12, 111–120

Ganswindt, A., Palme, R., Heistermann, M., Borragan, S., and Hodges, J.K., 2003. Non-invasive assessment of adrenocortical function in the male African elephant (*Loxodonta africana*) and its relation to musth. General and Comparative Endocrinology, 134, 156–166

Kataria, N., and Kataria, A., 2010. Can prolactin be a measurable marker of stress in dromedaries? Slovenian Veterinary Research, 47, 133–138

Mohamed, H.E., 2006. Factors affecting cortisol status in camels (*Camelus dromedarius*). Journal of Animal and Veterinary Advances, 5, 307–309

Mohammed, E., Fouad, R., Abdallah, S., El Hassan, T., Mohamed, F., Najia, E., Veronique, C., and Bernard, F., 2010. Effects of preslaughter stress on meat quality and phosphocalcic metabolism in camels (*Camelus dromedarius*). Journal of Camelid Science, 3, 33–38

Möstl, E., Messmann, S., Bagu, E., Robia, C., and Palme, R., 1999. Measurement of glucocorticoid metabolite concentrations in faeces of domestic livestock. Journal of Veterinary Medicine, 46, 621–31

Möstl, E., Maggs, J.L., Schrötter, G., Besenfelder, U., Palme, R., 2002. Measurement of cortisol metabolites in feces of ruminants. Veterinary Research Communication, 26, 127–139

Palme R., 2012. Monitoring stress hormone metabolites as a useful, non-invasive tool for welfare assessment in farm animals. Animal welfare, 21, 331–337

Palme, R., and Möstl, E., 1997. Measurement of cortisol metabolites in faeces of sheep as parameter of cortisol concentration in blood. International Journal of Mammalian Biology, 62, 192–97

Palme, R., Fischer, P., Schildorfer, H., and Ismail, M.N., 1996. Excretion of infused 14C-steroid hormones via faeces and urine in domestic livestock. Animal Reproduction Science, 43, 43–63

Rettenbacher, S., Möstl, E., Hackl, R., Ghareeb, K., and Palme, R., 2004. Measurement of corticosterone metabolites in chicken droppings. British Poultry Science, 45, 704–11

Saeb, M., Baghshani, H., Nazifi, S., and Saeb, S., 2010. Physiological response of dromedary camels to road transportation in relation to circulating levels of cortisol, thyroid hormones and some serum biochemical parameters. Tropical Animal Health and Production 42, 55–63

Touma, C., and Palme, R., 2005. Measuring fecal glucocorticoid metabolites in mammals and birds: the importance of validation. Annals of the New York Academy of Sciences, 1046, 54–74

Touma, C., Sachser, N., Möstl, E., and Palme, R., 2003. Effects of sex and time of day on metabolism and excretion of corticosterone in urine and faeces of mice. General and Comparative Endocrinology, 130, 267–278