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Monitoring the adrenocortical response to disturbances in sheep by measuring glucocorticoid metabolites in the faeces

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Summary

The determination of cortisol concentrations in plasma can be used as a parameter of animal welfare, but it is difficult not to influence the concentration of this hormone by the disturbance during fixation and blood collection. Non invasive monitoring, as for example by measuring the concentrations of faecal cortisol metabolites (FCM), avoids this problem. The aim of this study was primarily to get more information about the pattern of FCM during the time course of excretion and their stability after defaecation. Additionally we wanted to monitor manipulations of the sheep by measuring FCM.

In a first experiment we investigated, if the pattern of metabolites depends on the retention period of the metabolites in the sheep. Therefore the cortisol metabolites excreted via faeces collected at different times after an infusion of radioactive cortisol were analysed. The faecal samples were extracted and the radioactive FCM were separated by thin-layer chromatography and visualized using autoradiography. The results showed that most of the excreted metabolites were less polar than authentic cortisol, but more polar than progesterone. After peak excretion the relative amount of non-polar metabolites decreased compared to the more polar ones (metabolites with more hydroxy-groups). This can be explained by the enterohepatic circulation of the cortisol metabolites, which are hydroxylated most probably in the liver forming more and more polar compounds.

In a second experiment the stability of immunoreactive cortisol metabolites in sheep faeces was tested. 2 group specific enzyme immunoassays were used to quantify some of the metabolites. Assay A reacts with cortisol metabolites with a 5ß-11,17-dioxoandrostane structure and assay B with those with a 5ß-3α-hydroxy-11-oxo structure. After storing the faecal samples at room temperature for 1, 2, 4, 8 and 24 hours, respectively, the concentrations measured using assay A increased, whereas those recognised by assay B decreased. These results indicate that in sheep the faecal samples should be frozen soon after defaecation to get accurate results.

To monitor glucocorticoid excretion during routine handling procedures faecal samples from 37 female sheep (aged between 1 and 7 years) were collected after shear-

Schlüsselwörter: Stress, Radiometabolismus, Enzymimmunoassay, Kot, Transport, Schur.

Zusammenfassung

Schur und Transport verursachen beim Schaf eine vermehrte Ausscheidung von Glukokortikoidmetaboliten im Kot

Die Kortisolkonzentration im Blut ist ein Parameter für Belastungen. Es ist jedoch bei der Probennahme sicherzustellen, dass eine Fixierung des Tieres bzw. die Blutentnahme nicht ihrerseits zu einem Anstieg der Kortisolkonzentration führen. Eine Messung von Kortisolmetaboliten im Kot umgeht dieses Problem. Eine mögliche Beunruhigung der Schafe durch die Kotprobensammlung führt nicht zur Beeinflussung der Resultate, da die Glukokortikoidmetaboliten über die Galle in den Darm gelangen und somit die Ausscheidung über den Kot beim Schaf mit einer Verzögerung von ca. 12 Stunden erfolgt. Ziel unserer Arbeit war es, die Ausscheidung von Glukokortikoidmetaboliten über die Faeces bei Schafen nach der Schur bzw. nach dem Transport der Tiere zu messen. Im Rahmen der Methodenevaluierung untersuchten wir, ob das Metabolitenmuster von Kortisol im Kot von der Verweildauer im Körper abhängig ist. Nach der Infusion von radioaktivem Kortisol wurden Kotproben genommen (PALME et al., 1996), die Steroidhormonmetaboliten extrahiert und deren Zusammensetzung mittels Dünnschichtchromatographie/Autoradiographie getestet.

Die Ergebnisse zeigten, dass nur unkonjugierte Metaboliten im Kot ausgeschieden wurden, sich deren Zusammensetzung aber insofern änderte, als nach einem halben Tag nach der Applikation deutlich mehr polare Metaboliten ausgeschieden wurden als in der Anfangsphase der Elimination. Somit ist anzunehmen, dass es durch den enterohepatischen Kreislauf vermehrt zur Hydroxylierung der Metaboliten kommt.

Weiters wurde die Stabilität der immunreaktiven Kortisolmetaboliten untersucht. Für die Analyse wurden 2 verschiedene Immunoassays verwendet, von denen einer gegen 5ß-11,17-Dioxoandrostane (Assay A), der andere gegen 5ß-3α-hydroxy-11-Oxosteroide (Assay B) gerichtet war. Die Ergebnisse zeigten, dass die Konzentrationen der mit dem Assay A gemessenen immunreaktiven Kortisolmetaboliten während der Lagerung der Proben bei Raumtemperatur deutlich zunahmen, die mit Assay B gemessenen hingegen abnahmen.

ing and in addition from 5 female non pregnant sheep (between 2 and 4 years of age) during transport. The concentrations of glucocorticoid metabolites were measured using assay B. After shearing concentrations of FCM increased significantly ($p = 0.001$) from a median of 601 nmol/kg to 2,084 nmol/kg and after transport from 511 nmol/kg to 2,271 nmol/kg.

We conclude that the measurement of faecal cortisol metabolites can be used as a parameter to monitor adrenocortical activity during shearing and transport.

Abbreviations: a.m. = ante meridiem; ANOVA = analysis of variance; EIA = enzyme immunoassay; FCM = faecal glucocorticoid metabolites; p.m. = post meridiem; TLC = thin layer chromatography

Zur Messung der Glukokortikoidmetaboliten im Kot nach der Schur erfolgten Probennahmen bei 37 weiblichen Schafen (Alter zwischen 1 und 7 Jahre). Weiters wurden Proben bei einem Transport von 5 weiblichen, nicht trächtigen Schafen (Alter zwischen 2 und 4 Jahren, Transportdauer eine Stunde) gesammelt. Nach der Schur stieg die Konzentration der Glukokortikoidmetaboliten signifikant ($p < 0,001$) von einem medianen Basalwert von 601 nmol/kg Kot auf eine mediane Maximalkonzentration von 2.084 nmol/kg Kot an, nach dem Transport von 511 nmol/kg Kot auf 2.271 nmol/kg Kot.

Aus den Ergebnissen dieser Arbeit schließen wir, dass die Messung der Konzentration an Kortisolmetaboliten im Kot als Parameter für die Kortisolproduktion verwendet werden kann und dazu geeignet ist, Belastungen der Tiere durch landwirtschaftliche Maßnahmen zu quantifizieren.

Introduction

Assessing animal welfare is an innovative area of research involving new methods being devised and tested (MORMEDE et al., 2007). Comfortable handling and environment are of great importance for animal protection, but also for production goals, as disturbances can affect performance parameters such as milk yield, growth rate and reproduction (DOBSON et al., 2001). The questions of how to define animal welfare and how to measure it are still under discussion (FRASER, 2003). A potential indicator of animal welfare may be the detection of stress factors, but there is no standard definition of stress and no single biochemical assay system to measure it (HOFER and EAST, 1998).

Measurement of blood cortisol is an established method to quantify the reactions of animals to some stressors (TERLOUW et al., 1997). However, for this purpose repeated venipuncture is required, which is a source of stress by itself and can interfere with the results (HOPSTER et al., 1999). For this reason several non-invasive methods have been developed to quantify stress, such as measuring cortisol concentrations in saliva (FELL et al., 1985) or urine (HAY and MORMEDE, 1998). Both methods are established sampling procedures in humans but the interpretation is still under discussion (for review, see POLLARD, 1995). In lactating animals, the use of cortisol determination in milk was also described (HEMSWORTH et al., 2000). In lactating sheep regrouping and relocation was followed by a transient lower milk production with lower milk fat and milk protein content (SEVI et al., 2001). For avoiding disturbances of the animals and to increase the comfort of the animals, valid and reliable methods to identify and quantify potentially stressful situations are needed.

Among the 5 non-painful handling procedures of shearing, crutching, drenching, dipping and drafting, HARGREAVES and HUTSON (1990a) identified shearing as the most stressful manipulation by measuring the alterations of plasma cortisol and haematocrit. By monitoring changes in heart rate, plasma cortisol and haematocrit HARGREAVES and HUTSON (1990b) compared shearing to other potentially stressful events such as separation from other sheep, isolation, human presence, blood sampling, up-ending and exposure to shearing noise. Elevated heart rates were only detected during the procedure of shearing. Moreover, these authors found that shearing with wool removal induced a greater increase in heart rate, haematocrit and plasma cortisol than a similar procedure without wool removal.

In farm animals, saliva or urine sampling is laborious and also associated with disturbance of the animals. As cortisol is metabolised mainly in the liver and excreted not only via the urine but also via the bile (TAYLOR, 1971) the metabolites can be measured in the faeces. By infusing radioactive cortisol in sheep, LINDNER (1972) demonstrated the enterohepatic circulation of these metabolites. This author showed that in urine various conjugated C-21-metabolites are dominant in the early post-infusion time and were later replaced by 11-oxygenated androstane compounds. In sheep, PALME et al. (1996) described a delay time of about 12 hours between the infusion of radiolabelled cortisol and the maximum of radioactivity excreted via faeces. According to MÖSTL et al. (2002) faecal cortisol metabolites can be further metabolized by bacterial enzymes after defaecation. The measurement of these metabolites in the faeces has been successfully used to monitor disturbances in ruminants (PALME and MÖSTL, 1997; PALME et al., 1999; MÖSTL et al., 2002; PESENHOFER et al., 2006) and other species, such as horses (MÖSTL et al., 1999; GORGASSER et al., 2007). Faeces offer the advantage of an easy and feedback-free collection without unsettling the animals.

The aim of this study was primarily to get more information about the pattern of the faecal cortisol metabolites (FCM) during the time course of excretion and their stability after defaecation. Additionally we wanted to monitor manipulations of the sheep by measuring FCM.

Material and methods

Animals

For measuring the pattern of radioactive cortisol metabolites in relation to the time course of the excretion, faecal samples from an infusion experiment with ^{14}C cortisol (PALME et al., 1996) were used. For testing the stability of the immunoreactive metabolites after defaecation, fresh faecal samples from sheep were used.

Sampling after shearing and transport was conducted on the research farm of our University. These sheep were hardly accustomed to human manipulation and were kept in pens. The shearing was done during a practical course

Tab. 1: Antibodies, labels and standard used in the assays of faecal cortisol metabolites; both antibodies were raised in rabbits.

	Antibody against	Label	Standard
Assay A (PALME and MÖSTL, 1997)	5β-androstane-3α-ol-11,17-dione-3-HS:BSA	5β-androstane-3α-ol-11,17-dione-3-glucuronid:biotin	5β-androstane-3α-ol-11,17-dione (11-oxoaetiocholanolone)
Assay B (MÖSTL et al., 2002)	5β-androstane-3α-ol-11,17-dione-CMO:BSA	5β-androstane-3α-ol-11,17-dione-17-CMO:biotin	5β-androstane-3α-ol-11,17-dione (11-oxoaetiocholanolone)

Tab. 2: Multivariate analysis of influencing factors

Influencing factor	p - value
breed	0.188
gestation status	0.671
shearer	0.670
age	0.156
shearing duration	0.906

of handling sheep. 37 female sheep aged between 1 and 7 years were included in the shearing experiment. The group consisted of 3 breeds (20 milk-sheep, 11 stone-sheep, and 6 mountain-sheep). As allocation of voided faeces to individuals is difficult in a flock, but necessary for the interpretation of the results (TOUMA and PALME, 2005), all samples were taken directly from the ampulla of the rectum. In addition, samples were collected from 5 female non pregnant mountain sheep (between 2 and 4 years of age), which were transported for 1 hour. Faecal samples were collected immediately before and 6, 8, 10, 12 and 14 hours after both manipulations to study individual variations in delay time of the metabolite excretion. The sample taken at the beginning of shearing or before transport was considered to represent the baseline.

Chromatographic pattern of the faecal cortisol metabolites at different times after infusion of cortisol

Samples were extracted as described by PALME and MÖSTL (1997), using 80 % methanol. The methanolic extracts (1 ml) were transferred into another vial and evaporated to dryness. After redissolving in 50 µl methanol, the extract was spotted on a thin layer plate with fluorescence indicator (silica gel 60, F 254, Merck, Darmstadt, Germany). Non-radioactive cortisol and progesterone were used as markers for the polarity of the metabolites. The chromatogram was developed using 3 consecutive thin layer chromatograpy (TLC) runs (start to front distance 5, 10 and 15 cm, respectively) of trichloromethane/acetone mixtures of decreasing polarity (14 + 6, 16 + 4 and 18 + 2). The radioactivity was visualised using autoradiography on X-ray films, and the steroid standards were visualized under UV-light.

Influence of storing faeces at room temperature on the concentrations of immunoreactive substances

In a second experiment the stability of the immunoreactive cortisol metabolites in fresh faecal samples was tested. After defaecation, samples (n = 5) were incubated at room temperature for 1, 2, 4, 8 and 24 hours, respectively. The incubation was stopped by adding 80 % methanol and samples were stored at -24 °C until analysis using 2 different enzyme immunoassays. Assay A was described by PALME and MÖSTL (1997) and assay B by MÖSTL et al. (2002). Both assays used 11-oxoaetiocholanolone as standard, but the site of conjugation of the steroid to the carrier protein used for immunisation and the label differed (for details see Tab.1).

Shearing

To monitor glucocorticoid excretion during a routine handling procedure female sheep (n = 37, aged between 1 and 7 years) were sampled. The animals were selected out of the flock individually and led or carried to the place where they were sheared. Samples were taken directly after shearing and 6, 8, 10, 12 and 14 hours later. All faecal samples were frozen immediately after collection and stored at -24°C.

Shearing was done during a practical shearing-course for 11 students. Most of the students had no previous knowledge about shearing. Therefore the procedure took much longer than usual. To analyse the influence of the duration of shearing, we formed 3 groups (14 - 29 min, 30 - 44 min, 45 - 85 min shearing time). For reason of time it was not possible to shear all sheep on the same day. 24 animals were sheared on Day 1 and 13 on the following day. The extraction of samples was done as described by PALME and MÖSTL (1997). The analysis of FCM was performed using the assay B.

Transportation

The animals were separated in a pen and then caught individually in order to take the first samples before transport (9 a.m.). Subsequently, the animals were loaded onto the truck and transported for 1 hour on a winding, hilly route. After transport, the sheep were brought back to the pen again and further samples were collected in the same manner as in the shearing experiment at 3 p.m., 5 p.m., 7

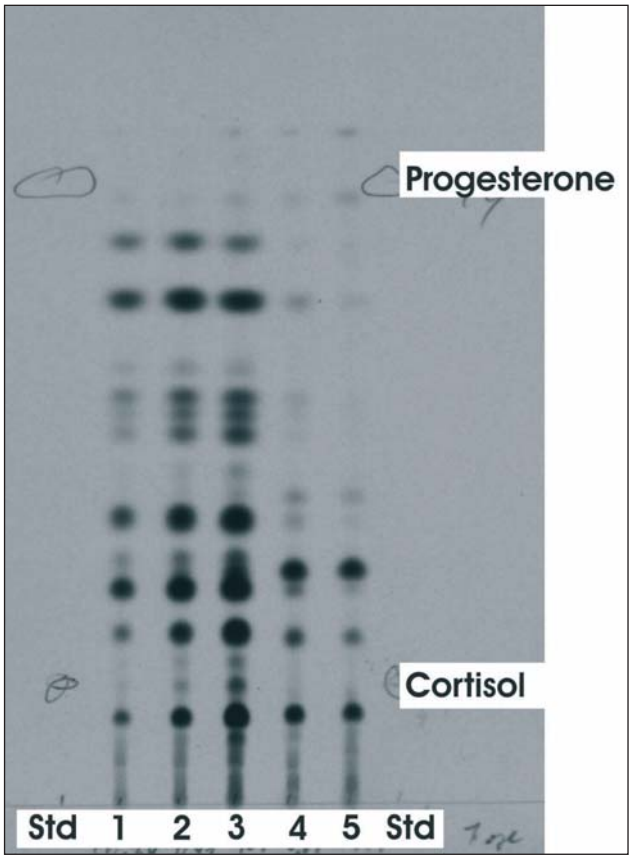

Fig.1: Autoradiography of ^{14}C faecal cortisol metabolites after thin layer chromatography; Std = standards (cortisol and progesterone)

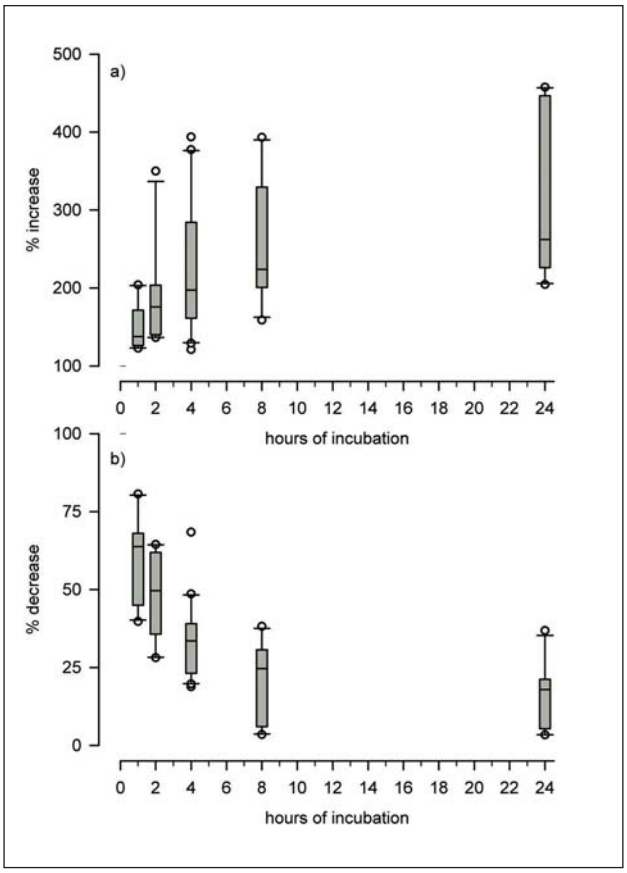

Fig. 2: Concentrations of faecal cortisol metabolites show a) an increase (measured with Assay A) or b) a decrease (measured with Assay B) during storing faeces at room temperature (100 % = concentration immediately after defaecation).

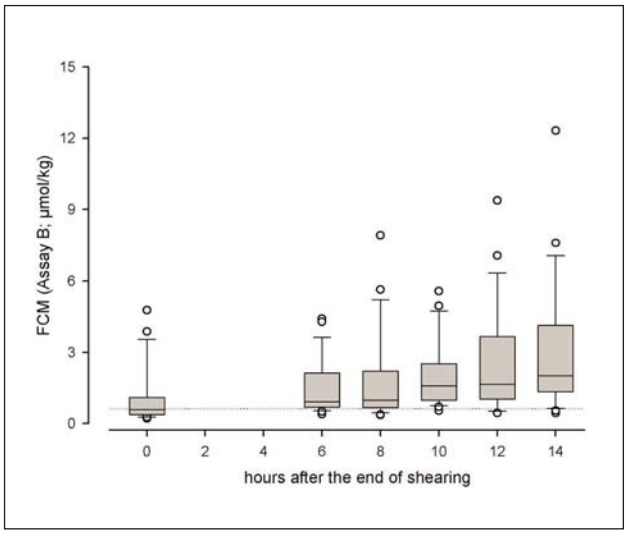

Fig. 3: Increase of the concentration of FCM (µmol/kg faeces) in all sheep caused by shearing on Day 1; results are shown as boxplot.

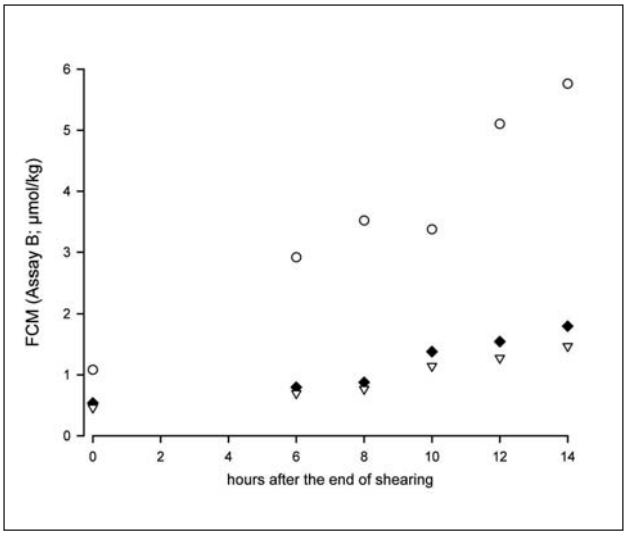

Fig. 4: Course of median FCM concentrations (µmol/kg faeces) of milk (diamond), stone (open circle) and mountain (triangle) sheep immediately and 6, 8, 10, 12 and 14 hours after shearing

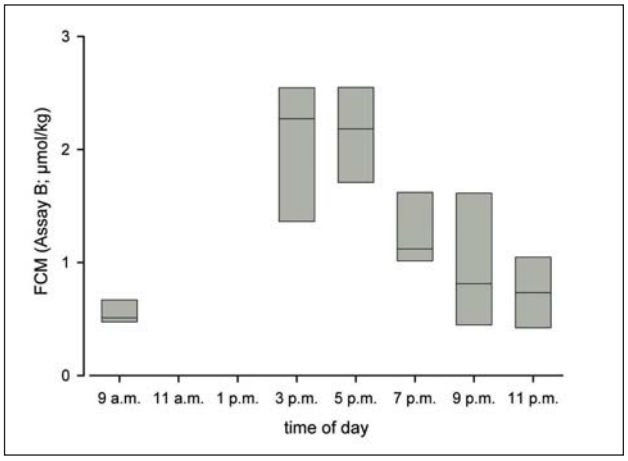

Fig. 5: Effect of transport of sheep on concentrations of FCM (µmol/kg faeces)

p.m., 9 p.m. and 11 p.m. All faecal samples were frozen immediately after collection and stored at -24 °C. Analysis of samples was conducted in the same way as in the shearing experiment.

Statistical analyses

Statistical analyses of the metabolite concentrations (increase of FCM, differences between breeds) were conducted by use of SPSS for Windows, version 14.0 respectively SigmaStat, version 3.5 and SigmaPlot, version 10.0 applying the Wilcoxon-Test for non-parametric paired samples or the Kruskal-Wallis-Test, respectively. To investigate the influence of possible confounders as breed, age, duration of shearing, shearer and gestation status concerning the shearing experiment the FCM values were subjected to a log-transformation and evaluated by ANOVA for repeated measures defining the above mentioned factors as covariates. A p-level of < 0.05 was considered as significant.

Results

Chromatographic pattern of the faecal cortisol metabolites at different times after infusion of cortisol

After autoradiography, a multitude of radioactive spots was seen on the TLC plate (Fig. 1). Most of the metabolites showed a polarity ranging between cortisol and progesterone. No substances with a faster chromatographic mobility than progesterone were detected. The chromatographic pattern of the metabolites changed after the maximum of excretion was exceeded. After half a day the excretion of the less polar cortisol metabolites was diminished and the more polar ones predominated.

Influence of storing faeces at room temperature on the concentrations of immunoreactive substances

Using immunoassay A the concentrations of immunoreactive FCM increased with prolonged storage (Fig. 2a) reaching peak concentrations after 24 hours. Contrary to this, the immunoassay B showed that the FCM measured decreased during the storage period and the lowest concentrations were measured after 24 hours (Fig 2b).

Shearing

The concentration of baseline values in animals sheared on Day 1 ranged from 221 to 4,773 (median: 601) nmol/kg faeces and peak levels ranged from 814 to 12,315 (median: 2,084) nmol/kg faeces (Fig. 3). The baseline values on Day 2 ranged from 376 to 3,227 (median: 1,238) nmol/kg faeces and peak levels ranged from 1,275 to 6,164 (median: 2,947) nmol/kg faeces.

The percentage increases in concentration of FCM from median baseline levels to median peak levels were 259 % in animals sheared on Day 1 and only 164 % in those on Day 2. To ensure that the percentage increase after shearing was not confounded by the arousal of the animals sheared on Day 2 by shearing the other animals on the day before (which may have caused the increased baseline concentrations on Day 2) the results of Day 2 were excluded from the statistical analysis.

On Day 1 peak concentrations of FCM appeared in half of the sheep within 14 hours after shearing (4 % after 6 hours; 8 % after 8 hours; 25 % after 10 hours and 12 % after 12 hours). In the other animals a decrease of the FCM after reaching peak concentrations was not observed during the sampling period of 14 hours. The significant overall increase of the concentration of FCM ($p = 0.001$) was not confounded by breed, age, duration of shearing, shearer or gestation status (Tab. 2) even though in univariate analysis those sheep that were in parturition at the day of shearing showed significantly higher values of FCM ($p < 0.001$). Anyhow, although there was no significant difference in the baseline values between the 3 breeds ($p = 0.143$) there was a significant ($p < 0.001$) higher increase in the FCM-values in the mountain sheep compared with the 2 other breeds that showed a comparable increase in the FCM values ($p = 0.984$; Fig. 4).

Transport

In this experiment, peak concentrations of FCM appeared 6 hours after transport. There was a statistically significant increase of FCM 6, 8 and 10 hours after transport ($p= 0.043$), whereas the FCM level 12 and 14 hours after transport did not differ from the baseline values ($p = 0.225$ and 0.345, respectively). Baseline values ranged from 452 to 751 (median: 511) nmol/kg faeces and peak level concentrations were between 2,182 and 2,785 (median: 2,271) nmol/kg faeces. This represents a median increase of 328 % from median baseline to peak values (Fig. 5), which was statistically significant ($p < 0.001$).

Discussion

Chromatographic pattern of the faecal cortisol metabolites at different times after infusion of cortisol

The results of the autoradiography showed that substances with the same chromatographic pattern as cortisol were not present in the samples and only cortisol metabolites were detected. This is in agreement with earlier findings of our group that authentic hormones are excreted via the faeces only in trace amounts if at all (MÖSTL et al., 2002; PALME et al., 2005). Radioactive steroid hormone metabolites are excreted during a relative long period and the polarity of the metabolites changed over time. This may be caused by the enterohepatic circulation of the radioactive metabolites (LINDNER, 1972), which cause the forma-

tion of more hydroxy-groups. This result shows that using assays strictly reacting with non-polar cortisol metabolites offer the possibility to get a sharper allocation of the increase of cortisol in blood as this group of metabolites is mainly excreted during the first 12 hours after infusion of radioactive cortisol.

Influence of storing faeces at room temperature on the concentrations of immunoreactive substances

In human faeces glucocorticoid metabolites are further metabolised to a great extent by bacterial enzymes in the gut (WINTER and BOKKENHEUSER, 1979). Assay A showed increasing concentrations with prolonged storage. This finding is in agreement with the results in cattle (MÖSTL et al., 1999) where a significant increase in the concentrations of immunoreactive metabolites was described within 1 hour incubation at room temperature using the same assay. In addition to bacterial metabolism also an aerial oxidation of the metabolites (EDMONDS et al., 2006) may be considered, but this explanation is less plausible to explain our results, as assay A is specific for the oxo-group at position 17 of the steroid molecule.

In our experiment using immunoassay B, values of immunoreactive cortisol metabolites decreased after storing the samples at room temperature. To explain these results a bacterial conversion (e.g. oxidation of the hydroxy-group at position 3 of the steroid molecule) was assumed. Due to this conversion the antibody is not able to detect the cortisol metabolites (MÖSTL et al., 2005), as it is specific for the position 3 and therefore the values decrease.

Shearing

The delay time between the peak of blood cortisol concentration and the appearance of maximum concentrations of its metabolites in faeces of sheep were described to be 10-12 hours, depending on the passage time of faeces through the gut (PALME et al., 1996). Therefore, faecal samples collected immediately after shearing or transport were considered as baseline values for the present analysis. It is one benefit of this type of non-invasive monitoring that it offers the advantage of a post hoc analysis.

Relatively large individual variations were detected in baseline as well as in peak values of FCM. This is in accordance with the findings of PALME et al. (1999) showing a great inter-animal variability in basal values (range: 93 to 1,031 nmol/kg faeces) and in peak values (range: 445 to 3,622 nmol/kg faeces). Thus for evaluating disturbances PALME et al. (1999) and TOUMA and PALME (2005) recommend to include either a large number of animals in the experiment or to use each animal for its own control by expressing the increase of FCM as percentage of particular basal values.

As individual attribution of the faecal samples was needed, it was necessary to take all faecal samples directly from the ampulla of the rectum. The values of the individual FCM were used for subgroup analysis concerning breed, age, gestation status as well as the effect of shearing duration and shearer.

The comparison of baseline concentrations of animals sheared on the first day of the shearing course to those sheared on Day 2 showed that the median level calculated from the first samples taken on Day 2 was twice as high as on Day 1. This difference was probably caused by disturbances of the herd during catching the sheep for shearing and sampling at the first day. The increase between baseline and peak values on Day 2 was lower (164 %) than on Day 1 (259 %), which is also an indicator that this group had already elevated initial values at the beginning of sampling.

The higher baseline concentrations measured in our experiment in comparison with the results described by PALME et al. (1999) can be explained by the use of another EIA (assay B). This assay showed also higher concentrations in cows, as it cross-reacts with more cortisol metabolites (MÖSTL et al., 2002).

The highest FCM concentrations were measured in half of the animals within 14 hours after shearing, whereas in the other sheep FCM levels were still increasing during the sampling period. Therefore it is uncertain if these values measured 14 hours after shearing represent the concentration maxima. A further increase of concentration of FCM cannot be excluded. In this study the delay time between the disturbing event and the maximum of FCM excretion was longer than reported by PALME et al. (1999). These authors described that after ACTH infusion, peak concentrations were reached after about 10 hours, but they also detected variations in delay time ranging from 6.0 to 18.7 hours. The passage time may vary because of the amount of food available. ADAMS et al. (1994) stated that after the infusion of ^{3}H-oestradiol-17β in sheep, the passage time of digesta through the gut was slower in food-restricted ewes than in ewes getting additional food. An explanation for the delayed appearance of peak concentrations of FCM in this study could be that the sheep ate less as they were agitated due to the shearing and therefore had a longer gut passage time.

The subgroup analysis on breeds revealed that in mountain-sheep the median concentration throughout all sampling times were about 3 times higher compared to the others (stone-sheep: 954 nmol/kg faeces; milk-sheep: 1,128 nmol/kg faeces; mountain-sheep: 3,447 nmol/kg faeces). This observation is rather surprising, as stone-sheep appeared to be more scared due to the procedure of shearing and taking faecal samples than the other breeds. The differences in glucocorticoid production between breeds may be caused by a selection of different coping styles as for example described in territorial birds (great tit; *Parus major*), which were selected according to their coping strategies (CARERE et al., 2003). The authors investigated 2 lines of birds (slow and fast explores), and described higher corticosterone metabolite excretion via droppings after stress in slow compared to fast responders.

In the sheep which gave birth on the day of experiment, the concentration of FCM was obviously higher than in the other sheep (basal values: 1,332 to 3,877 nmol/kg faeces; peak values: 7,583 to 12, 314 nmol/kg faeces), which could be interpreted as an effect of parturition. However, as all the sheep, giving birth on the day of shearing were mountain-sheep one has to consider that the elevated values of FCM can be caused by breed affiliation rather than by parturition, which corresponds with the result of the multivariate analysis (Tab. 2).

Although KILGOUR and LANGEN (1970) found higher concentrations of plasma cortisol after slow shearing than after conventional shearing, the current study did not find any influence of shearing duration or shearers on the concentration of FCM. However, one has to consider that in this study the shearing procedure was part of a practical

course for sheep handling for students and the very long durations for shearing were caused by the lack of experience of the students.

In this shearing experiment there was 1 sheep with an especially high basal (4,773 nmol/kg faeces) and peak (5,872 nmol/kg faeces) value. As this sheep was a milk-sheep, these high values cannot be explained by breed affiliation. It can be assumed that the high basal values originate either from an unknown disturbance before the beginning of the experiment (for example hierarchy conflicts), or this sheep suffered from some non obvious pain. The evaluation including this mentioned sheep showed that in the group of milk-sheep a statistically significant increase of FCM from basal value could only be found 14 hours after shearing. If the mentioned sheep is excluded there would be a significant rise in the concentration of FCM after 6, 10, 12 and 14 hours. Perhaps the specific order of dominance hierarchy of the sheep could be another influencing factor for the great variance of basal values. Therefore the hierarchy level of each animal should be determined in further studies.

Transport

Unlike in the shearing experiment, during transport all samples were taken at the same time as intervals starting at the same time of day. In cattle, PALME et al. (2000) and MÖSTL et al. (2002) measured the highest concentrations of FCM in cows about 12 hours after transport. In the current study the maximum concentrations of FCM already appeared after 6 to 8 hours and returned approximately to baseline levels 14 hours after transport.

The relatively distinct increase of FCM from baseline to peak values of 327 % suggests that transportation causes higher cortisol production compared to shearing. With regard to the rapid decrease of the FCM peak concentrations to baseline values the disturbance of transport seems to be of short duration. This finding could be related to the fact that the sheep were transported for 1 hour only and then brought back to their familiar environment. To the contrary, MÖSTL et al. (2002) reported elevated concentrations of FCM up to 5 days after transport in cows, which were brought to a new environment.

Conclusion

This study demonstrated that it is possible to quantify disturbances caused by routine handling procedures by measuring the concentration of cortisol metabolites in the faeces of sheep. The identification and quantification of disturbances is a precondition to improve housing conditions and management procedures. Therefore, measuring FCM can serve as a valuable non-invasive tool in further studies to optimise animal welfare and economic results in sheep.

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