Urinary Cortisol Levels in Japanese Shorthorn Cattle before and after the Start of a Grazing Season

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ABSTRACT : We conducted two experiments to assess the effect of transfer from housing to grazing on stress hormone secretion in cattle using urine samples. In a preliminary experiment, urine samples were collected following an adrenocorticotrophic hormone (ACTH) challenge, and cortisol levels in urine were compared with the levels in plasma. In a second experiment, urinary cortisol was measured before and after the start of a grazing season in 6 Japanese Shorthorn cows, all of which had experienced grazing before. In experiment 1, urinary cortisol showed a pattern of changes similar to that of plasma with a 0.5-h temporal lag time, and the peak levels were 4 to 10 times higher than the basal levels. In experiment 2, the urinary cortisol levels in cows did not change after the cows were let out to pasture, with no decreases in body weight. This study suggests that the transfer from housing to grazing did not affect physiological responses to cause high excretion of urinary cortisol in grazing-experienced cattle using a non-invasive sampling method.
(Asian-Aust. J. Anim. Sci. 2005. Vol 18, No. 10 : 1430-1434)

Key Words : Cortisol, Urine, Stress, Cattle

INTRODUCTION

The grazing period in Japan, except in part of the southern area, is usually limited by climatic conditions to the period from April to November. After being transferred from housing to pasture, cattle often show negative performances, such as decreases in body weight, diarrhea, or nematode parasite infections. These lost productivities that occur after the transfer from housing to pasture are caused by environmental changes, e.g., changes in feeding and weather conditions. In regard to the microbial population in the rumen, Oshio et al. (1981) have reported that it takes over 3 weeks to adapt to a grazing condition. It is conceivable that these changes also affect physiological responses.

Several studies have demonstrated that cattle have behavioural and physiological stress responses to being tethered after grazing (Ladewig and Smidt, 1989; Redbo, 1993; Morrow et al., 2000; Morrow et al., 2002); however, the grazing adaptation during the transfer from housing to pasture has not been studied in depth.

Recently, animal welfare is of increasing public interest, and the absence of chronic stress is one of its prerequisites (Möstl and Palme, 2002). In mammals, stress is usually assessed by the level of plasma cortisol, which is secreted by the adrenal gland in response to the activity of the hypothalamic-pituitary-adrenal (HPA) axis. As for chronic stress, prolonged cortisol elevations have been detected in lying-restricted cows (Fisher et al., 2002). However, capturing and blood sampling are themselves known to cause a rise in the cortisol levels (Morton et al., 1995; Hopster et al., 1999). Therefore, some researchers have investigated non-invasive sampling procedures such as the determination of cortisol or cortisol metabolites in urine (Miller et al., 1991; Redbo, 1993; Hay et al., 2000; Morrow et al., 2000), feces (Miller et al., 1991; Möstl et al., 1999; Wasser et al., 2000; Morrow et al., 2002), saliva (Leeuw et al., 2003), or milk (Verkerk et al., 1998; Wenzel et al., 2003) using farm, zoo, and wildlife animals.

The objectives of our study were therefore to (1) assess the use of urinary cortisol to monitor adrenal activity in cattle using a commercially available kit, and (2) investigate the effects of the transfer from housing to grazing on urinary cortisol levels as a means of assessing the perception of stress levels.

MATERIALS AND METHODS

All experiments were performed at National Agricultural Research Center for Tohoku Region, where cattle routinely are let out to pasture in late April and are brought inside in late October.

Experiment 1

Animals and procedures : Three multiparous Japanese Shorthorn cows (595±51 kg live weight) were used in the adrenocorticotrophic hormone (ACTH) challenge test. They were kept in tethers and fed twice a day at 9:00 and 16:00 h with a standard concentrate diet (1 kg/day) and grass silage

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Received September 26, 2004; Accepted February 26, 2005

Table 1. Mean plasma cortisol concentrations and integrated adrenocortical responses following the administration of saline (n = 3) or adrenocorticotrophic hormone (ACTH, n = 3) to cattle

Item	Saline	ACTH	SEM[1]
Cortisol (ng/ml)			
At -30 min	4.10	4.72	0.74
At 6 h	5.81	5.04	1.65
At 36 h	7.77	5.93	2.13
Integrated cortisol response (ng·min/ml[2])	2,492[a]	8,673[b]	389

[1] Standard error of least squares means.

[2] Area under the plasma cortisol concentration×time curve over 6 h.

[a, b] Means within a row with different superscripts are significantly different ($p<0.01$).

(8 kg dry matter (DM)/day). The concentrate diet consisted mainly of flaked corn, corn gluten feed, wheat bran, rice bran, rapeseed meal, and soybean meal. Each cow was fitted with an indwelling jugular catheter 2 h before the start of the experiments. A dose of 40 IU of ACTH (Sigma, St. Louis, MO, USA) diluted in 2 ml saline was injected twice at a 2-h interval via the catheter according to the protocol that reliably elevates plasma cortisol concentrations for 4 to 6 h with a low dose of ACTH and as few injections as possible (Verkerk et al., 1994). For the control, saline (2 ml) was injected in the same way as the ACTH. The interval between the ACTH and saline administrations was at least 1 week.

Sampling : Blood samples were taken at -30, 0, 30, 60, 90, 120, 150, 180, 210, 240, 270, 300, 330, 360 min and 12, 24, 48 h with administration following the 0 and 120 min samples. Blood samples were collected into heparinised tubes and centrifuged, and the plasma was stored at -20°C until analysis for cortisol concentration. All urine samples from spontaneous urination were collected from each cow during the blood samplings. A sub-sample of the collected urine sample (30 ml) was filtered to remove debris, and stored at -20°C until being assayed for cortisol and creatinine concentrations.

Experiment 2

Animals and procedures : Animals were studied from 2 weeks before to 4 weeks after they were let out to pasture in spring 2003. Six Japanese Shorthorn cows aged 3 to 10 years, with a mean live weight of 653 kg (range: 594 to 722 kg) at the beginning of the study, were used in this experiment. The animals were housed in a pen, in which they were given free access to a neighboring pine woods (2.5 ha) for another experiment not shown here, after a previous grazing period. During the housing period, the animals were fed twice a day at 09:00 and 15:30 h. The amount of concentrate was 1 kg per animal per day, while the amount of roughage (consisting of hay) on average was 12.5 kg DM. Water was available *ad libitum*. All of the animals were moved to pastures consisting mainly of Kentucky bluegrass (*Poa pratensis* L.) on May 1 2003 and were transferred to fresh pastures on a 2-week basis. The pastures had a total area of 4 ha. Three samples of 0.25 m^2 plots in randomly distributed grazing exclusion cages, clipped at approximately 5 cm above ground level, were collected at the beginning and at the end of each grazing cycle. Additionally, 5 samples of 0.25 m^2 plots outside of each cage were collected for an estimate of forage availability.

Sampling : Spontaneously voided urine samples from each animal were collected once a day, at days 14, 7, 6 and 3 before the start of grazing. Additional samples were collected from days 1 to 7, and at days 14, 21 and 28 after the grazing started. Urine sampling was conducted during the day (9:00 to 15:00 h). Urine samples were handled as described in Experiment 1. The animals were weighed every week during the study.

Assays

Plasma and urine cortisol concentrations were analyzed using a commercially available immunoassay kit (Neogen, Corp., Lexington, KY, USA). The assay was validated for bovine urine by performing parallelism and recovery tests. The dilution curve of bovine urine exhibited a parallelism with the standard curve. The recovery of exogenous cortisol added in known amounts to bovine urine was 86%. The inter- and intra-assay coefficients of variation were 10.4% and 7.0%, respectively. Urinary cortisol concentrations were divided by urinary creatinine to correct for urine dilution (Klante et al., 1997). Creatinine levels were determined by spectrophotometry (Creatinine test Wako, Wako Chemical, Inc., Osaka, Japan). Accordingly, urine cortisol levels were expressed as ng cortisol/mg creatinine.

Statistical analyses

Differences between treatments were analyzed by the GLM procedure of the SAS (1988), in which blocks were formed on individual animals. To determine whether the variation of cortisol levels during the experiment was significant, the statistical model included day and animal. Correlations between plasma and urinary cortisol levels at each subsequent time point were evaluated to estimate the lag time using Pearson's correlation coefficient as calculated by the CORR procedure of the SAS.

RESULTS

Experiment 1

The mean values of the adrenocortical response variables of animals subjected to ACTH or saline are shown in Table 1. There were no differences in plasma cortisol concentrations at -30 min, 6 h, and 36 h after the initial injection between the ACTH and saline groups, but the calculated areas under the plasma cortisol concentrations×

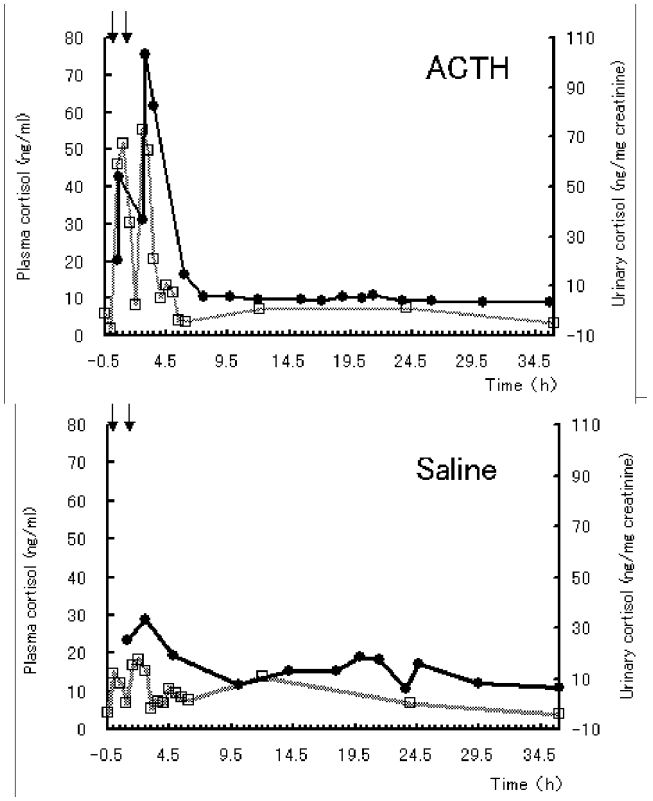

Figure 1. Concentrations of plasma cortisol (open square) and urinary cortisol (closed circles) in a representative cow following the administration of ACTH or saline at time 0 and 2 h (indicated by arrows).

time curve over 6 h were much greater in animals subjected to ACTH than in the controls ($p<0.01$).

Figure 1 shows the cortisol response to ACTH or saline administration in a representative animal. Both the first and the second ACTH administrations caused rapid increases in plasma cortisol concentrations (<0.5 h) and decreases in the basal levels within 2 h after each administration in all three animals, although we used the procedure in which the elevation of plasma cortisol levels was expected to be sustained for 4 to 6 h as described by Verkerk et al. (1994). Urinary cortisol levels showed a similar pattern of changes to plasma cortisol. The lag time between the peak plasma cortisol and elevated urinary cortisol levels was 0.5 h, with high coefficients of correlation between these parameters ($r = 0.897$, $p<0.01$).

Figure 2 shows the mean levels of urinary cortisol during 6-h periods following the administration of ACTH or saline to cattle. In animals subjected to ACTH, the urinary cortisol level during the first 6-h period was 4 to 10 times higher than those measured during the other periods ($p<0.01$). There was no significant change in the urinary cortisol levels following the saline treatment.

Experiment 2

Figure 3 shows the changes in mean urinary cortisol

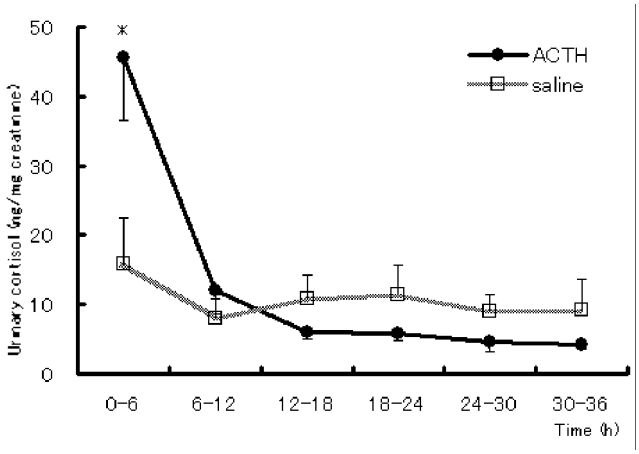

Figure 2. Mean concentrations of urinary cortisol during 6-h periods in cattle following the administration of ACTH (closed circles) or saline (open square) at time 0 and 2 h. Values are means ±standard errors. * indicates significant difference from other 6-h periods within the same treatment ($p<0.01$).

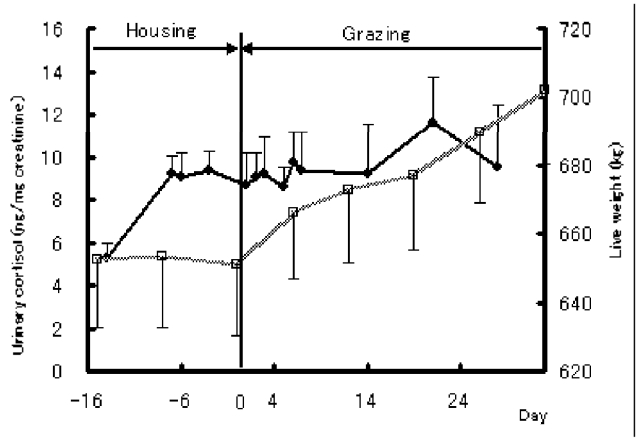

Figure 3. Mean urinary cortisol levels (closed circles) and live weight (open square) before and after the start of the grazing season. Values are mean±standard errors. Day 0 indicates the start of grazing.

levels and live weight in 6 animals before and after the start of the grazing season. Live weight increased after the start of grazing, and the mean live weight at day 33 after the cows were let out to pasture was significantly higher than those at day 0 (702 vs. 651 kg, respectively, $p<0.01$). The average available forage was 25.9 kg DM/animal in the grazing condition. In the urinary cortisol levels, no significant variation was detected around the start of grazing, although the level tended to be low at day -14. Mean urinary cortisol levels during 1 week did not differ between before and after the grazing began (8.84 vs. 9.12 ng/mg creatinine, respectively).

DISCUSSION

The basal urinary cortisol level in the present study (4.3 to 12 ng/mg creatinine) was lower than that found in

Friesian cows by Morrow et al. (2000: 53 ng/mg creatinine). That could be partly explained by the difference of the cattle breed in addition to the difference of sensitivity of the antibody in the assay between these studies. However, the good parallelism and relative highly percentage of recovery in our study indicate that the commercial ELISA kit used in this study measures not absolute but relative cortisol levels in bovine urine.

The ACTH challenge test showed that urinary cortisol is responsive to exogenous ACTH, as it peaked 0.5 h later than in plasma. This time lag measured in the present study is similar to those previously reported using cows (1 h; Morrow et al., 2000), sheep (2 h; Miller et al., 1991), and pigs (1.5 h; Hay et al., 2000). Except for this time lag, the profile for urinary cortisol was similar to the changes observed in plasma. Blood sampling requires physical restraint and causes physiological stress (Morton et al., 1995; Hopster et al., 1999). This results show that urine sampling can be a non-invasive alternative to plasma sampling as a method for monitoring HPA axis function after the ACTH challenge, and that the commercial kit is available for urinary cortisol in cattle.

In this study, urinary cortisol was almost constant around the start of the grazing season, and the averaged levels during the housing and grazing conditions were not different, indicating no adrenocortical stress response to cause high excretion of urinary cortisol. In regard to the transfer from pasture grazing to being housed indoors, urinary cortisol levels were increased for heifers the day after tethering but declined a week later (Redbo, 1993). Similar results have been found in plasma cortisol from bulls (Ladewig and Smidt, 1989) and urinary cortisol from cows (Morrow et al., 2000) in response to tethering and restraint. These results suggest that a restricted condition involving tethering in stalls causes stress, and that afterward physiological down-regulation occurs. The absence of variation of the urinary cortisol levels around the start of grazing observed in our study might be caused by the absence of tethering during the pre-grazing. Moreover, it might be caused by the fact that our cattle had free access to a neighboring pine woods during the pre-grazing. It has also been reported that the heifers showed a significant increase in time spent on stereotypies during post-grazing compared with that during pre-grazing (Redbo, 1990). This finding might imply that environmental changes could be more stressful for cattle after the grazing period than before.

Japanese Shorthorn has a higher roughage intake and higher tolerance toward Japanese theileriosis than European breeds (Saeki, 1993). In addition, all animals used in the present study had experienced grazing before this study. Although decreases in body weight, diarrhea, or nematode parasite infection often occur in cattle after they are transferred from housing to pasture, our cows showed higher roughage intake and body weight gain after the grazing began compared with pre-grazing. It could therefore be concluded that the environmental changes from housing to grazing for our Japanese Shorthorn cattle did not cause physiological stress sufficient to activate the HPA axis.

In conclusion, the cortisol level in urine, in spite of the relative values detected using a commercial ELISA kit, seems to be a viable alternative to the cortisol level in plasma with a non-invasive sampling method for cattle. Due to increasing public interest in organic farming, including animal welfare, animal production systems based on grazing are drawing attentions worldwide, especially in Japan. To avoid negative performances just after the start of grazing, it is necessary to understand the physiological changes that occur in animals that are transferred from housing to pasture. In this study, it has been shown that no changes occurred in urinary cortisol before and after the start of grazing using animals that had previously experienced grazing. However, several factors seem to have affected our results, such as pre-grazing condition, grazing experience, or amount of pasture. Further studies are required to obtain a precise understanding of the effect of the transfer to grazing on physiological responses in conjunction with animal behaviour.

ACKNOWLEDGEMENTS

The authors thank Dr. S. Kushibiki, Dr. N. Takenouchi for their technical advices, and they are very grateful to the staff of the Pasture and Grazing Management Section and Ms. Sakamoto for their technical assistances.

REFERENCES

Fisher, A. D., G. A. Verkerk, C. J. Morrow and L. R. Matthews. 2002. The effects of feed restriction and lying deprivation on pituitary-adrenal axis regulation in lactating cows. Livest. Prod. Sci. 73:255-263.

Hay, M., M.-C. Meunier-Salaün, F. Brulaud, M. Monnier and P. Mormède. 2000. Assessment of hypothalamic-pituitary-adrenal axis and sympathetic nervous system activity in pregnant sows through the measurement of glucocorticoids and catecholamines in urine. J. Anim. Sci. 78:420-428.

Hopster, H., J. T. N. van der Werf, J. H. F. Erkens and H. J. Blokhuis. 1999. Effects of repeated jugular puncture on plasma cortisol concentrations in loose-housed dairy cows. J. Anim. Sci. 77:708-714.

Klante, G., T. Brinschwitz, K. Secci, F. Wollnik and S. Steinlechner. 1997. Creatinine is an appropriate reference for urinary sulphatoxymelatonin of laboratory animals and humans. J. Pineal Res. 23:191-197.

Ladewig, J. and D. Smidt. 1989. Behavior, episodic secretion of cortisol, and adrenocortical reactivity in bulls subjected to tethering. Horm. Behav. 23:344-360.

Leeuw, J. A., E. D. Ekkel, A. W. Jongbloed and M. W. A. Verstegen. 2003. Effects of floor-feeding and the presence of a foraging substrate on the behaviour and stress physiological

response of individually housed gilts. Appl. Anim. Behav. Sci. 80:297-310.

Miller, M. W., N. T. Hobbs and M. C. Sousa. 1991. Detecting stress responses in Rocky Mountain bighorn sheep (*Ovis canadensis canadensis*): reliability of cortisol concentrations in urine and feces. Can. J. Zool. 69:15-24.

Morton, D. J., E. Anderson, C. M. Foggin, M. Kock and E. Tiran. 1995. Plasma cortisol as an indicator of stress due to capture and translocation in wildlife species. Vet. Rev. 136:60-63.

Morrow, C. J., E. S. Kolver, G. A. Verkerk and L. R. Matthews. 2002. Fecal glucocorticoid metabolites as a measure of adrenal activity in dairy cattle. Gene. Comp. Endocrinol. 126:229-241.

Morrow, C. J., E. S. Kolver, G. A. Verkerk and L. R. Matthews. 2000. Urinary corticosteroids: an indicator of stress in dairy cattle. Proc. N.Z. Soc. Anim. Prod. 60:218-221.

Möstl, E., S. Messmann, E. Bagu, C. Robia and R. Palme. 1999. Measurement of glucocorticoid metabolite concentrations in faeces of domestic livestock. J. Vet. Med. A46:621-631.

Möstl, E. and R. Palme. 2002. Hormones as indicators of stress. Domest. Anim. Endocrinol. 23:67-74.

Oshio, S. and I. Tahata. 1981. Studies on the method of adaptation to grazing environment: 2. Influence of various feeds before grazing on the microbial populations in the rumen of calves grazed on pasture (in Japanese, with Japanese abstract). Bull. Natl. Grassl. Res. Inst. 20:132-144.

Redbo, I. 1990. Changes in duration and frequency of stereotypies and their adjoining behaviours in heifers, before, during and after the grazing period. Appl. Anim. Behav. Sci. 26:57-67.

Redbo, I. 1993. Stereotypies and cortisol secretion in heifers subjected to tethering. Appl. Anim. Behav. Sci. 38:213-225.

Saeki, H. 1993. A present state of study of Japanese theileriasis (in Japanese). J. Anim. Protozooses 4:64-73.

SAS Institute Inc. 1988. SAS User's Guide (Release 6.03). SAS Institute Inc., Cary, North Carolina.

Verkerk, G. A., K. L. Macmillan and L. M. McLeay. 1994. Adrenal cortex response to adrenocorticotropic hormone in dairy cattle. Domest. Anim. Endocrinol. 11:115-123.

Verkerk, G. A., A. M. Phipps, J. F. Carragher, L. R. Matthews and K. Stelwagen. 1998. Characterization of milk cortisol concentrations as a measure of short-term stress responses in lactating dairy cows. Anim. Welfare 7:77-86.

Wasser, S. K., K. E. Hunt, J. L. Brown, K. Cooper, C. M. Crockett, U. Bechert, J. J. Millspaugh, S. Larson and S. L. Monfort. 2000. A generalized fecal glucocorticoid assay for use in a diverse array of nondomestic mammalian and avian species. Gene. Comp. Endocrinol. 120:260-275.

Wenzel, C., S. Schönreiter-Fischer and J. Unshelm. 2003. Studies on step-kick behavior and stress of cows during milking in an automatic milking system. Livest. Prod. Sci. 83:237-246.