Asian-Aust. J. Anim. Sci.
Vol. 22, No. 10 : 1437 - 1440
October 2009

www.ajas.info

Urinary Catecholamine and Cortisol Responses of Japanese Shorthorn Cows to Social Isolation

Y. Higashiyama*, M. Nashiki[1] and H. Narita[2]
National Agricultural Research Center for Tohoku Region, Akahira 4,
Shimo-kuriyagawa, Morioka, Iwate, 020-0198, Japan

ABSTRACT : This study was performed to investigate the use of urinary catecholamines to monitor changes in the activity of the sympathetic nervous system and to determine the relationship of urinary cortisol and catecholamines in Japanese Shorthorn cows in response to social isolation. One cow was isolated from its group, which consisted of 14 cows (457 to 756 kg BW, 2 to 12 years old), for three days. The isolated cow was in contact with the other cows visually only at meal times. This isolation was repeated for 6 cows. Spontaneously voided urine samples were collected from the experimental animals once a day, before the treatment and on days 1, 2, and 3. Urinary cortisol and adrenaline levels were significantly increased compared with pre-isolation levels on the first day, and then declined to the basal levels during the next two days. Urinary noradrenaline levels changed in the same way as cortisol and adrenaline levels, but the difference was not significant. Urinary cortisol levels tended to be correlated with those of urinary adrenaline, but not noradrenaline. This study suggests that the urinary adrenaline levels can be a non-invasive indicator of stress and that the change of urinary adrenaline is similar to that of urinary cortisol. (**Key Words :** Urinary Cortisol, Urinary Catecholamines, Social Isolation)

INTRODUCTION

In mammals, the hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic nervous system (SNS) are the two main systems activated in response to stress, resulting in increased secretion in blood of cortisol (HPA axis) and catecholamines (SNS). Unfortunately, capturing and blood sampling themselves are known to cause a rise in these levels. In addition, the secretion of these hormones exhibits pulsative and circadian variation, which can lead to difficulties in the blood-sampling procedure. Urine is the main elimination route of these hormones and can be collected non-invasively. Moreover, these urinary hormones accumulate over several hours. Therefore, the cortisol level in the urine has been used to monitor HPA activity in several farm species, including pigs (Hay et al., 2000), sheep (Miller et al., 1991), and cattle (Higashiyama et al., 2007). In regard to SNS, it is possible to determine catecholamines and their metabolites in urine in pigs (Hay et al., 2000; Foury et al., 2007). Dehnhard (2007) has shown that changes in urinary catecholamine excretion reflect fluctuations in sympathoadrenal activity in elephants. To our knowledge, however, there has been no study about urinary catecholamines in cattle, except for post-slaughter investigations (Lowe et al., 2004).

Cattle have been demonstrated to be sensitive to various acute stressors. In particular, social isolation is known to induce struggling and significant increases in vocalization, heart rate, and plasma cortisol concentrations, suggesting severe psychological stress (Boissy and Le Neindre, 1997; Rushen et al., 1999). The objectives of our study are i) to assess the use of urinary catecholamines to monitor changes in the activity of SNS, and ii) to investigate the relationship of urinary cortisol with urinary catecholamines, in cattle in response to social isolation.

MATERIALS AND METHODS

Animals, treatment, and urine sampling

Six Japanese Shorthorn cows were used as experiment animals. The group consisted of 14 cows (457 to 756 kg BW, 2 to 12 years old). All animals were housed in a pen, from which they were given free access to a neighboring pine woods (2.5 ha). The animals were fed twice a day

* Corresponding Author: Yumi Higashiyama. Tel: +81-19-643-3562, Fax: +81-19-643-3562, E-mail: kawakita@affrc.go.jp
[1] National Institute of Livestock and Grassland Science, 768 Senbonmatsu, Nasu-shiobara, Tochigi, 329-2793, Japan.
[2] Sapporo, 065-0041, Japan.
Received February 12, 2009; Accepted June 18, 2009

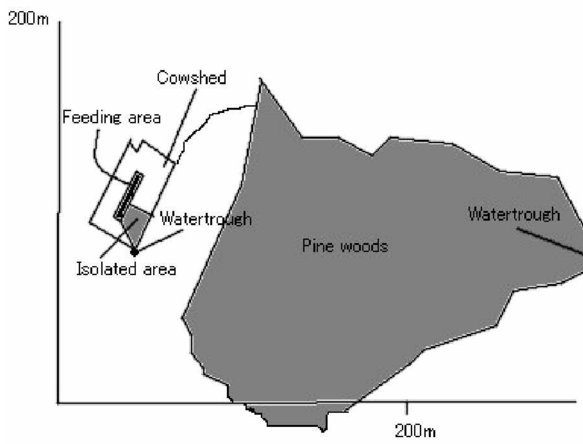

Figure 1. Experimental facilities to study the effect of social isolation in cattle.

(09:00 and 16:00 h). The amount of concentrate was 1 kg per animal per day, while the roughage (consisting of hay) was provided *ad libitum*. The animals had free access to water.

The experiment animals were subjected to social isolation wherein one cow was isolated from its group and prevented by an electric fence from accessing the pine woods for three days. The isolation on the first day was started at 08:30 h. All animals except the isolated one spent all day from meal to meal in the pine woods, which were invisible from the isolated area (Figure 1).

Spontaneously voided urine samples were collected from the experiment animals once a day, before the treatment and on days 1, 2, and 3. An experimenter collected urine in a tube as soon as the animals spontaneously urinated. Urine sampling was conducted at the first urination after 09:00 h, except that it was conducted 2 h after the isolation treatment on the first day, because urinary cortisol has been shown to display a pattern of changes similar to that of plasma with a temporal lag time (Higashiyama et al., 2005).

Cortisol and catecholamines analyses

Urine cortisol and catecholamine (adrenaline and noradrenaline) concentrations were analyzed using immunoassay kits (Oxford Biomedical Research, Inc., Oxford, MI, USA; Labor Diagnostika Nord, Nordhorn, Germany). The inter- and intra-assay coefficients of variation for cortisol, adrenaline, and noradrenaline analyses were 10.7% and 5.2%, 9.0% and 11.0%, and 11.6% and 13.0%, respectively. These concentrations in urine were divided by urinary creatinine levels to correct for urine dilution. Creatinine levels were determined by spectrophothometry (Creatinine-test-Wako, Wako Chemical, Inc., Osaka, Japan).

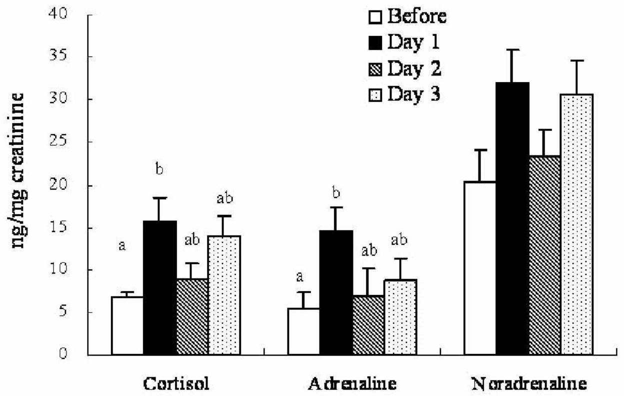

Figure 2. Urinary cortisol and catecholamine levels before and after social isolation. Values are means±SE. Different letters indicate significant differences ($p<0.05$).

Statistical analysis

Statistical analyses were carried out using the statistical package SAS 9.13 (SAS Institute Inc., Cary, NC, USA). The data were subjected to the Shapiro-Wilk test for normality. Differences between pre- and post-isolation were analyzed by Scheffe's multiple range test (Scheffe, 1959), in which blocks were formed on individual animals using the GLM procedure. Correlations between the data were obtained using Peason's correlation coefficient as calculated by the CORR procedure.

RESULTS AND DISCUSSION

The start time of sampling was delayed for 1.5 h on day 1 compared with the other sampling days. However, that delay is not considered because the time range was as small as that of spontaneous urination of each experiment animal.

Urinary cortisol and adrenaline levels were significantly increased compared with pre-isolation levels on the first day, and then declined to the basal levels during the next two days (Figure 2). Urinary noradrenaline levels also tended to increase on day 1 ($p<0.07$). Previous studies have shown that short-term social isolation increases cortisol secretion (e.g., Rushen et al., 1999). However, this increase seems to disappear when social isolation is prolonged (Munksgaard and Simonsen, 1996), suggesting physiological down-regulation or adaptation to the new environment. Our results indicate that social isolation caused a transient increase of urinary cortisol levels in cattle.

In contrast to cortisol secretion, however, relatively few studies have characterized catecholamine responses to stress in domestic animals, partly because plasma catecholamines have been regarded as a highly variable experimental end point (Minton, 1994). However, Odore et al. (2004) reported that transportation significantly induced transient increases in blood catecholamine levels in calves,

Table 1. Correlation coefficients among urinary hormone levels in cows

	Adrenaline	Noradrenaline
Cortisol (ng/mg creatinine)	0.375[1]	0.088
Adrenaline (ng/mg creatinine)		0.173
Noradrenaline (ng/mg creatinine)		

[1] Approaching significance ($p<0.08$).

suggesting endocrine modifications induced by stress conditions. In addition, Lowe et al. (2004) showed that pre-slaughter handling affected urinary adrenaline levels in postmortem bulls. The results of the present study indicate that stress affects urinary catecholamine levels in living cattle also.

Table 1 lists the correlations among urinary hormone levels. Urinary cortisol levels tended to be correlated with those of urinary adrenaline, but not noradrenaline. These results are similar to those of Foury et al. (2005), who reported that cortisol and adrenaline levels were highly correlated, much more than cortisol and noradrenaline levels measured in pig urine collected after slaughter. In their study, urinary adrenaline levels were also highly correlated with urinary noradrenaline levels. Another study reported by Foury et al. (2007) indicated that the concentrations of all three stress hormones in urine were inter-correlated. In our study, however, there was no correlation between urinary adrenaline and noradrenaline levels. Apple et al. (1995) reported that restraint and isolation stress elicited dramatic elevations in plasma levels of cortisol and adrenalin, but not in noradrenaline in sheep. Adrenaline is particularly considered to be an indicator of mental stress, whereas increased noradrenaline levels seem to be mainly related to increased physical activity (Odore et al., 2004). In addition, Foury et al. (2005) pointed out that the adrenal cortex (cortisol) and medulla (adrenaline) are co-activated, but that the HPA axis (cortisol) and the SNS (noradrenaline) are largely independent. These mechanisms may be related with positive correlation only between urinary cortisol and adrenaline levels in our study. In addition, the relatively few animals in our study may affect the weak correlation between levels of noradrenaline and cortisol, or adrenaline in urine. Further studies using numerous animals are required to obtain a precise understanding of the respective influences of these hormones.

In conclusion, the present study has demonstrated that urinary adrenaline levels temporarily increased in response to social isolation in Japanese Shorthorn cows and that the change of urinary adrenaline was similar to that of urinary cortisol. Our results indicate that urinary adrenaline may be a non-invasive indicator of stress as urinary cortisol in both slaughtered and living cattle. Smulders et al. (2006) showed that salivary cortisol and urinary catecholamines were linked with behavioural aspects related to animal welfare using pigs. Our results using a non-invasive technique will contribute to animal welfare as well as to animal physiology.

ACKNOWLEDGMENT

The authors are very grateful to the staff of the Pasture and Grazing Management Section and Ms. M. Sakamoto for their technical assistance.

REFERENCES

Apple, J. K., M. E. Dikeman, J. E. Minton, R. M. McMurphy, M. R. Fedde, D. E. Leith and J. A. Unruh. 1995. Effects of restraint and isolation stress and epidural blockade on endocrine and blood metabolite status, muscle glycogen metabolism, and incidence of dark-cutting longissimus muscle of sheep. J. Anim. Sci. 73:2295-2307.

Boissy, A. and P. Le Neindre. 1997. Behavioral, cardiac and cortisol responses to brief peer separation and reunion in cattle. Physiol. Behav. 61:693-699.

Dehnhard, M. 2007. Characterisation of the sympathetic nervous system of Asian (*Elephas maximus*) and African (*Loxodonta Africana*) elephants based on urinary catecholamine analyses. Gen. Comp. Endocrinol. 151:274-284.

Foury, A., N. Devillers, M.-P. Sanchez, H. Griffon, P. Le Roy and P. Mormède. 2005. Stress hormones, carcass composition and meat quality in Large White×Duroc pigs. Meat Sci. 69:703-707.

Foury, A., N. A. Geverink, M. Gil, M. Gispert, M. Hortós, M. Font I Furnols, D. Carrion, S. C. Blott, G. S. Plastow and P. Mormède. 2007. Stress neuroendocrine profiles in five pig breeding lines and the relationship with carcass composition. Animal 1:973-982.

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.

Higasihyama, Y., H. Narita, M. Nashiki, M. Higashiyama and T. Kanno. 2005. Urinary cortisol levels in Japanese Shorthorn cattle before and after the start of a grazing season. Asian-Aust. J. Anim. Sci. 18:1430-1434.

Higashiyama, Y., M. Nashiki, H. Narita and M. Kawasaki. 2007. A brief report on effects of transfer from outdoor grazing to indoor tethering and back on urinary cortisol and behaviour in dairy cattle. Appl. Anim. Behav. Sci. 102:119-123.

Lowe, T. E., C. E. Devine, R. W. Wells and L. L. Lynch. 2004. The relationship between postmortem urinary catecholamines, meat ultimate pH, and shear force in bulls and cows. Meat Sci. 67:251-260.

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.

Minton, J. E. 1994. Function of the hypothalamic-pituitary-adrenal axis and the sympathetic nervous system in models of acute stress in domestic farm animals. J. Anim. Sci. 72:1891-1898.

Munksgaard, L. and H. B. Simonsen. 1996. Behavioral and pituitary adrenal-axis responses of dairy cows to social isolation and deprivation of lying down. J. Anim. Sci. 74:769-778.

Odore, R., A. D'Angelo, P. Badino, C. Bellino, S. Pagliasso and G. Re. 2004. Road transportation affects blood hormone levels and lymphocyte glucocorticoid and β-adrenergic receptor concentrations in calves. Vet. J. 168:297-303.

Rushen, J., A. Boissy, E. M. C. Terlouw and A. M. B. de Passillé. 1999. Opioid peptides and behavioral and physiological responses of dairy cows to social isolation in unfamiliar surroundings. J. Anim. Sci. 77:2918-2924.

Scheffe, H. 1959. The analysis of variance. John Wiley & Sons, Inc., NY.

Smulders, D., G. Verbeke, P. Mormède and R. Geers. 2006. Validation of a behavioral observation tool to assess pig welfare. Physiol. Behav. 89:438-447.