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Milk cortisol response to group relocation in lactating cows

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The aim of the study reported in this Research Communication was to analyse the variations of milk cortisol concentrations in response to the relocation of dairy cows between production groups. Milk cortisol measured during 3 consecutive days did not vary significantly in cows without environmental perturbation. However, relocation of cows caused a significant increase of cortisol in milk starting from the first milking after the group change. This suggests that cortisol in milk can be a suitable biomarker to assess the HPA response of dairy cows to a short/medium-term environmental challenge.

Keywords: Dairy cow, milk cortisol, intra-day variation, mild stressor, relocation.

Cortisol is the primary effector molecule of the hypothalamic–pituitary–adrenal (HPA) axis involved in the response to stressors (Kirovski et al. 2014). The determination of cortisol in blood is a widely used method (Sgorlon et al. 2015), but for cows it is a highly invasive technique that may induce activation of the HPA axis, increasing plasma cortisol level (Rushen et al. 2008).

It is known that steroid hormones can permeate cell membranes and may cross the epithelial blood-milk barrier of mammary gland alveoli (Rushen et al. 2008), showing a high correlation between milk and plasma cortisol concentrations after ACTH administration (Thinh et al. 2011). Considering that milking for dairy cows is a routine process, milk might be a non-invasive alternative to blood in bovine HPA axis evaluation. However, the analysis of milk cortisol in field conditions is necessary before it could be claimed as a reliable biomarker of an animal's ability to cope with environmental stimuli. Therefore, in the present study the variations in milk cortisol concentrations in response to relocation stress were analysed.

Materials and methods

The measure of variations of milk cortisol concentrations in Holstein Friesian (HF) and Norwegian Red (NR) cows exposed or not to perturbations was performed in two experiments. In study 1 the inter-day variations of cortisol concentration were evaluated. For this, milk samples were collected for 3 consecutive days from 20 cows during morning (6:00 AM) and afternoon (6:00 PM) milking. In study 2, cows were relocated from the Post-partum group to the group of Fresh cows (relocation PF), from the group of Fresh cows to the High production group (relocation FH) and from the High production group to the Low production group (relocation HL). Milk samples from these animals were collected individually once a day during the evening milking (6:00 PM) for five consecutive days, starting from 2 d before the relocation, which occurred on the morning of day 3. Cortisol in milk was analysed as reported by Gabai et al. (2006). Detailed information about animals, experimental procedures, ethical statement and statistical analysis are reported in the Supplementary File as online Supplementary Materials and Supplementary Table S1.

Results and discussion

In study 1, when no apparent environmental perturbation occurred, significant variations of milk cortisol concentrations between- and within-day were not observed (Fig. 1, online Supplementary Table S2). Despite the demonstration that environmental conditions and management can affect the circadian rhythm of cortisol secretion others (Mormede et al. 2007; Ogino et al. 2014) did not detect a rhythm in cortisol release in well fed, free ranging cows, while they observed a clear circadian rhythm in cows under tie-stall and restricted feeding conditions. Under our experimental conditions, as in most commercial farms, animals were free ranging and nutritional requirements were adequately met. Likely, these conditions did not favour a circadian rhythm of cortisol release and, considering that milk cortisol reflects plasma free cortisol variations between two consecutive milking (Shutt & Fell, 1985), this led to the lack of

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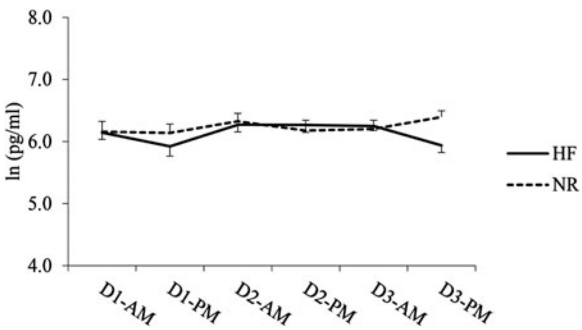

Fig. 1. Effect of breed, day of sampling and time of sampling on cortisol concentration in milk (ln of pg/ml) in Study 1. Milk samples were collected in three consecutive days (D1, D2, D3) at 6:00 AM and 6:00 PM. HF, Holstein Frisian; NR, Norwegian Red; AM – before noon milk sampling; PM – after noon milk sampling; Variations in milk cortisol concentrations within day, between days of sampling and between breeds resulted to be not significant.

difference in milk cortisol concentrations between morning and afternoon milking. However, these observations cannot be generalised and within-day cortisol release should be also analysed under different environmental and farming situations.

The significant increase ($P < 0{\cdot}01$) in milk cortisol concentrations on days 3, 4 and 5 (Table 1), observed in Study 2, suggests that relocation is a trigger for the HPA axis in the bovine species. Indeed, Gupta et al. (2005) observed a significant increase in plasma cortisol in steers after repeated regrouping and relocation. In lactating cows, the increase in plasma cortisol induced by relocation is the most likely explanation for the subsequent increase in milk cortisol.

The differences in milk cortisol concentrations following the relocations can hardly be explained by differences in milk yield. Indeed, milk yield was affected ($P < 0{\cdot}01$) by variables related to the stage of lactation (production group). On the contrary, none of the variables related to the stage of lactation showed any effect on milk cortisol concentrations. Fukasawa & Tsukada (2010) reported that fresh cows showed significantly higher milk cortisol concentrations in comparison to mid and late lactating cows, thus suggesting that the stage of lactation can affect milk cortisol concentrations. However, in the study of Fukasawa & Tsukada (2010) the group of fresh cows included animals between 7 and 90 DIM (days in milking), while in Study 2 of this paper all animals in the PF group were beyond 50 DIM.

In addition, our previous observations (Gabai, unpublished data), indicate that milk cortisol concentrations are significantly higher ($P < 0{\cdot}01$) in the first week postpartum ($1{\cdot}52 \pm 0{\cdot}14$ ng/ml), decrease by the second week of lactation ($0{\cdot}86 \pm 0{\cdot}12$ ng/ml) and then remain stable as far as day 30 after parturition ($0{\cdot}83 \pm 0{\cdot}12$ ng/ml). Moreover, potential effects on milk cortisol related to mammary inflammation can be excluded, as SCC in our animals was lower than 200 000 cells/ml and an increase in milk cortisol concentration can be only observed when SCC is higher than 400 000 cells/ml (Sgorlon et al. 2015). Thus, relocation was the prominent stimulus for HPA activation and milk cortisol increase.

Milk yield showed a significant ($P < 0{\cdot}01$) increase on day 3 followed by a decrease on days 4 and 5 (Table 1). The transient increase in milk yield could be explained by the positive effect of cortisol on glycaemia (Sgorlon et al. 2012), possibly related to increased peripheral gluconeogenesis. However, this temporary increase in milk yield lasted only 1 d and then decreased. The effect of cortisol on milk yield is controversial, as most information derives from exogenous ACTH/glucocorticoids administration (van der Kolk, 1990). However, stress and glucocorticoids may affect milk yield by different mechanisms (Silanikove et al. 2000).

Despite the two breeds being present on the same farm under the same environmental conditions, breed differences in milk cortisol were not observed in either study. Neither Parity nor the Breed × Parity interaction influenced milk cortisol concentrations. However, we observed that the extent of the response to the environmental challenge was very variable among cows and, in our opinion, this observation deserves further consideration. Here, the reduced number of animals does not allow us to identify subpopulation of cows with different response intensity. In a previous work (Sgorlon et al. 2015), we observed a significant difference in milk cortisol concentrations between Italian Holstein and Italian Simmental cows and hypothesised that the different ability to cope with milk yield could explain, at least in part, that difference. It is important to consider that, in that previous work, milk cortisol was measured in animals belonging to fourteen farms, and that one breed only was present in each farm. Indeed, mean milk cortisol concentrations were different among farms and Holstein herds did not necessarily show the higher cortisol concentrations. This observation suggests that different environmental conditions can affect the 'basal' HPA axis activity, and superimpose the genetic background (Mormede et al. 2007).

Up to now, the limited amount of data available on milk cortisol concentrations does not allow the identification of a threshold to define cows' wellbeing. In cattle, the function of the HPA axis is not well characterised. Studies on rhythmicity, responses to pharmacological treatments and to chronic stress led to inconsistent results and available data are far from conclusive (Mormede et al. 2007). Therefore, more studies are needed for better understanding of both environmental and genetic factors affecting milk cortisol concentrations. However, based on the results of this work, cortisol measurement in milk samples collected during consecutive days can be considered as a suitable biomarker to assess the individual HPA response of dairy cows to the same short/medium-term environmental challenge and the overall conditions of the herd.

Further studies are required to investigate if milk cortisol can be useful for prediction of animals with an increased risk of developing diseases and to aid the selection of more resilient animals.

Table 1. Effects of breed, group of lactation, day of sampling and parity on milk cortisol concentration and milk yield in Study 2

	ln (pg/ml)	SE		Milk yield (kg)	SE	
Breed						
NR	6·13	0·12	ns	32·80	2·31	ns
HF	6·02	0·08	ns	30·80	1·53	ns
Group						
PF	6·23	0·09	ns	37·98	1·55	A
FH	6·08	0·15	ns	31·33	2·80	B
HL	5·92	0·10	ns	26·03	1·79	C
Day of sampling						
D1	5·85	0·11	B	32·02	1·31	B
D2	5·76	0·11	B	31·56	1·32	B
D3	6·21	0·10	A	33·16	1·35	A
D4	6·31	0·09	A	30·35	1·31	C
D5	6·38	0·10	A	30·67	1·34	C
Parity						
Primiparous	6·03	0·06	ns	31·91	1·16	ns
Pluriparous	6·12	0·14	ns	31·66	2·59	ns
Breed × Day of sample			0·121			0·742
Group × Day of sample			0·237			0·000
Parity × Breed			0·094			0·258
Breed × Group × Day of sample			0·850			0·378

HF, Holstein Frisian; NR, Norwegian Red; PF: from postpartum to fresh cows; FH: from fresh to high production cows; HL: from high production to low production cows; Primiparous: first calving cows; Pluriparous: second and third calving cows; ns: not significant; values within columns with different letters A, B, C differ significantly, $P < 0·01$.

Supplementary material

The supplementary material for this article can be found at https://doi.org/10.1017/S0022029916000790.

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Competing interests

The authors declare that they have no competing interests.

Authors' contributions

N. P. participated to the study design, sample collection, data analysis and manuscript preparation; G. G. participated in drafting the manuscript; B. S. participated in conceiving the study and drafting the manuscript; L. D. D. carried out the immunoassays; S. S. participated in conceiving and designing the study, collecting samples and had major responsibilities in data analysis and drafting the manuscript.

References

Fukasawa M & Tsukada H 2010 Relationship between milk cortisol concentration and the behavioral characteristics of postpartum cows introduced to a new group. *Animal Science Journal* **81** 612–617

Gabai G, Mollo A, Marinelli L, Badan M & Bono G 2006 Endocrine and ovarian responses to prolonged adrenal stimulation at the time of induced CL regression. *Reproduction in Domestic Animals* **41** 485–493

Gupta S, Earley B, Ting STL & Crowe MA 2005 Effect of repeated regrouping and relocation on the physiological, immunological, and haematological variables and performance of steers. *Journal of Animal Science* **83** 1948–1958

Kirovski D, Snoj T, Nedic S, Nedic D, Cebulj-Kadunc N & Kobal S 2014 Non-invasive methods for detecting chronic stress in dairy cows. In *Proceedings of the First DairyCare Conference*, p. 46 (Ed. C Knight). DairyCare COST Action FA1308 ISBN 978-0-9930176-0-5

Mormede P, Andanson S, Auperin B, Beerda B, Guemene D, Mamkvist J, Manteca X, Manteuffel G, Prunet P, van Reenen CG, Richard S & Veissier I 2007 Exploration of the hypothalamic-pituitary-adrenal function as a tool to evaluate animal welfare. *Physiology and Behavior* **92** 317–339

Ogino M, Matsuura A, Yamazaki A, Irimajiri M, Suzuki Y, Kushibiki S, Singu H, Kasuya E, Hasegawa Y & Hodate K 2014 Plasma cortisol and prolactin secretion rhythms in cattle under varying environments and management techniques. *Animal Science Journal* **85** 58–68

Rushen J, de Passillé AM, von Keyserlingk MAG & Weary DM 2008 *The Welfare of Cattle Publisher*. Netherlands: Springer

Sgorlon S, Colitti M, Asquini E, Ferrarini A, Pallavicini A & Stefanon B 2012 Administration of botanicals with the diet regulates gene expression in peripheral blood cells of Sarda sheep during ACTH challenge. *Domestic Animal Endocrinology* **43** 213–226

Sgorlon S, Fanzago M, Guiatti D, Gabai G, Stradaioli G & Stefanon B 2015 Factors affecting milk cortisol in mid lactating dairy cows. *BMC Veterinary Research* **11** 259

Shutt DA & Fell R 1985 Comparison of total and free cortisol in bovine serum and milk or colostrum. *Journal of Dairy Science* **68** 1832–1834

Silanikove N, Shamay A, Shinder D & Moran A 2000 Stress down regulates milk yield in cows by plasmin induced beta-casein product that blocks K + channels on the apical membranes. *Life Science* **67** 2201–2212

Thinh NC, Yoshida C, Long ST, Yusuf M & Nakao T 2011 Adrenocortical response in cows after intramuscular injection of long-acting adrenocorticotropic hormone (tetracosactide acetate zinc suspension). *Reproduction in Domestic Animals* **46** 296–300

Van der Kolk JH 1990 The bovine pituitary-adrenocortical axis and milk yield. *Veterinary Quarterly* **12** 114–120