RESPONSE TO ACUTE EMOTIONAL STRESSORS AND ENDOCRINE STIMULATION IN DAIRY COWS AND HEIFERS

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INTRODUCTION

Dairy cows experience a number of potentially stressful situations, such as being handled by humans, being transported or left alone, as part of their everyday life. There is evidence for genetic and individual variation in approachability and temperament (review by Burrows, 1997) and other behaviour related traits in cattle (LeNeindre *et al.* 1995). The ability of individuals to cope with stressors has shown genetic variation in laboratory species (*e.g.* mice, Popova *et al.* 1993). The response to stressors involves activation of the hypothalamus-pituitary-adreno-axis (HPA-axis). Milk production involves a number of endocrine systems, which interact with the HPA-axis. As dairy cattle populations are subjected to intensive selection for production, a correlated response in traits of the HPA-axis is obviously possible. The aim of this study was to examine the genetic variation in the HPA-axis' response of dairy cattle when 1) isolated in an unfamiliar environment (social isolation), 2) handled, and 3) subjected to *i.v.* injection with corticotropin releasing factor (CRF). The responses were measured as plasma concentrations of cortisol and adrenocorticotropic hormone, ACTH.

MATERIALS AND METHODS

Cows and heifers. A total of 355 Holstein cows and heifers of the Future Genetics nucleus herd in Denmark were tested. Their ancestors were traced as far back as possible, giving a total of 3109 animals in the pedigree. Cows were kept in a loose-housing system with cubicles and slatted walking areas. Heifers were tethered.

Design. Animals were tested as heifers (age 362 ± 15 d), and as cows early (60 ± 11 d after calving) and late (165 ± 14 d after calving) in their first lactation. Animals were blocked into batches tested at the same time. Each animal was subjected to three tests: 1) Led by halter for approximately 20 meters for cows and 50 meters for heifers to social isolation for 15 minutes (SI), 2) led by halter by personnel over a distance equal to that of the SI test (handling, H), and 3) an intravenous CRF stimulation test (CRF). Order of testing was randomly assigned to batches. Results of 86 batches are included in this report.

Testing protocol. Prior to testing, cows were restrained at the eating area (crushes). Stalls and crushes are referred to as home environment, where animals were at the start of each test. One animal at a time was subjected to SI or H. At the SI test, the animal was led to an animal trailer covered with a tarpaulin and restrained. Isolation lasted for 15 minutes, after which the animal was led back to its home environment. Blood was sampled at –2, 17, 30 and 45 minutes relative to start of isolation. The H test was similar to the SI test. However, the isolation period was substituted by a 15 minutes period in the home environment. The CRF test was carried out in the home environment. Corticotropin releasing factor (synthetic bovine CRF, Bachem, Bubendorf, Switzerland) was administered *i.v.* at 0.1 μg/kg body weight dissolved in sterile NaCl solution, as a bolus dose. Blood was sampled at -15, 0, 15, 30, 45 and 90 minutes relative to CRF administration.

Blood was collected using venipuncture (Vacuette, Greiner, Germany), stabilized with heparin or EDTA, and cooled on ice. Plasma was harvested by centrifugation (2000 *g*, 4°C, 15 minutes) and stored frozen (-20°C) until assayed for cortisol and ACTH within 30 days after sampling. ACTH was assayed using a sandwich type assay based on monoclonal antibodies and detection of time resolved fluorescence (Dobson *et al.*, 1987). Cortisol was assayed using a commercial assay kit (PerkinElmer Life Sciences, Turku, Finland).

Statistical methods. Concentrations of ACTH or cortisol were log-e transformed before further analysis. From each test, a baseline value (prior) was derived, either from the single sample taken prior to test or as the mean of the two samples prior to CRF administration. At the H and SI test an estimated concentration at 15 minutes was obtained using linear regression lines through the points at 15, 30, and 45 minutes (t = 15). For the CRF test a fitted value for t = 30 minutes was calculated. Furthermore, the area below the response curve between 0 and 90 minutes was calculated as the sum of the trapezoid area (AUC). Variables for ACTH and cortisol were both calculated according to the above rules. Bivariate mixed models were fitted to two variables within response hormone of each test, one always being the prior concentration:

$$\mathbf{y} = \mathbf{X}\boldsymbol{\beta} + \mathbf{Z_b b} + \mathbf{Z_p p} + \mathbf{Z_a a} + \mathbf{e} \qquad (1)$$

where $\mathbf{y}$ was a vector of two log-e transformed plasma concentrations of ACTH or cortisol, $\boldsymbol{\beta}$ was a vector of the fixed effect age group, **b**, **p**, **a**, and **e** were vectors of the random effects batch, animal (permanent), animal (genetic), and residual error, respectively, $\mathbf{X}$, $\mathbf{Z_b}$, $\mathbf{Z_p}$, and $\mathbf{Z_a}$ were incidence matrices for age group, batch, animal (permanent) and animal (genetic), respectively. Heritability, h^2, was calculated as $\mathbf{h}^2 = \sigma_a^2/(\sigma_a^2 + \sigma_p^2 + \sigma_e^2)$ and repeatability, t, was calculated as $\mathbf{t} = (\sigma_a^2 + \sigma_p^2)/(\sigma_a^2 + \sigma_p^2 + \sigma_e^2)$ using variance components estimated with an AI-REML algorithm included in the DMU package (Jensen and Madsen, 2000).

RESULTS

The heifers had higher prior concentrations of ACTH and cortisol than the cows at either stage of lactation. The heifers also responded stronger (Figure 1) to all treatments than the cows did. Response profiles of ACTH and cortisol followed almost similar time courses. Handling only caused a small elevation in ACTH concentration above prior levels in either of the age groups (Figure 1). However, a clear response to handling was observed for cortisol in the heifers. The ACTH and cortisol responses to social isolation were of a magnitude similar to that of the CRF test in heifers, but smaller in cows of both age groups. The repeatabilities of ACTH variables were moderate for all traits, whereas the cortisol traits had low repeatabilities (Table 1). The response variables tended to have a higher repeatability than the prior value for the same test. An exception was the H test, where there was very low response.

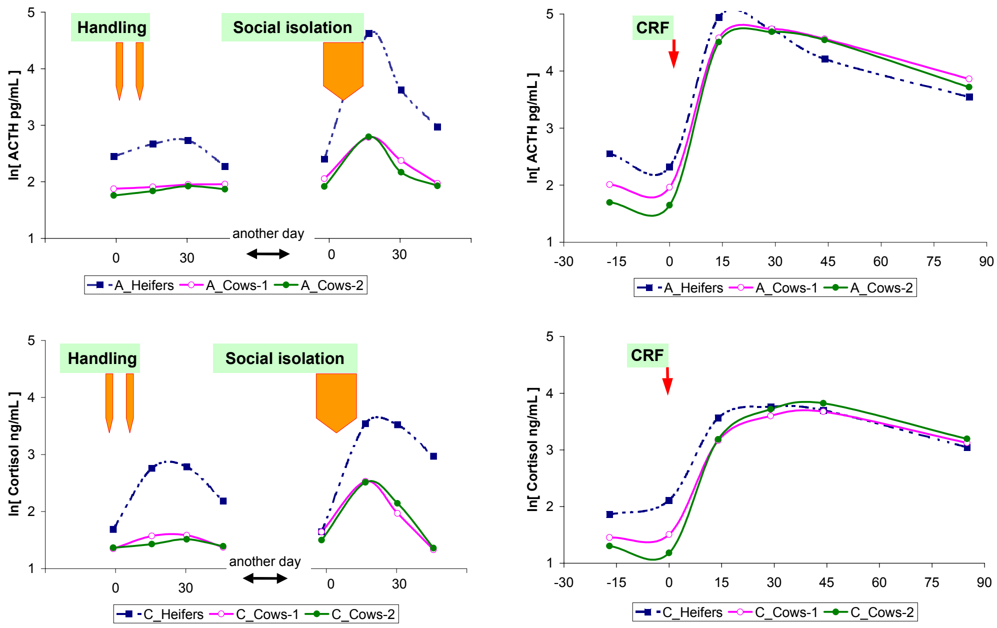

Figure 1. Response profiles at handling, social isolation, and CRF-challenge in heifers, early laction cows (Cows-1), and late lactation cows (Cows-2). Response is plasma ACTH (upper panel) and plasma cortisol (lower panel).

Table 1. Parameters[A] estimated for heritability, repeatability, and genetic and phenotypic correlation between plasma concentrations in three different tests.

		Repeatability, t		Heritability, h^2		Phenotypic corr., r_p	
Test	Variable	ACTH	Cortisol	ACTH	Cortisol	ACTH	Cortisol
CRF	Prior	$0.27_{0.10}$	$0.13_{0.09}$	$0.01_{0.14}$	$0.04_{0.12}$	0.20	-0.07
	AUC	$0.36_{0.11}$	$0.23_{0.12}$	$0.10_{0.14}$	$0.23_{0.13}$		
CRF	Prior	$0.21_{0.10}$	$0.13_{0.09}$	$0.01_{0.14}$	$0.04_{0.12}$	0.14	-0,01
	t = 30	$0.29_{0.11}$	$0.16_{0.10}$	$0.14_{0.13}$	$0.15_{0.11}$		
Social isolation	Prior	$0.41_{0.15}$	$0.01_{0.09}$	$0.26_{0.15}$	$0.01_{0.12}$	0.35	0.12
	t = 15	$0.53_{0.13}$	$0.14_{0.12}$	$0.17_{0.14}$	$0.14_{0.13}$		
Handling	Priorl	$0.35_{0.13}$	$0.06_{0.09}$	$0.06_{0.15}$	$0.02_{0.13}$	0.57	0.36
	t = 15	$0.23_{0.14}$	$0.02_{0.10}$	$0.13_{0.16}$	$0.01_{0.14}$		

[A]Standard errors in subscript.

Heritabilities for ACTH and cortisol response variables were low to moderate, and heritabilities for most prior variables were low (Table 1). Phenotypic correlations between concentrations of ACTH or cortisol prior to test and subsequent response were in general low, except for the H test, where the response was small (Table 1).

DISCUSSION

This study has shown that HPA-axis response to standardised stimulation by *i.v.* administered releasing hormone, and emotional stress from social isolation are repeatable traits in dairy cattle, and traits, that show additive genetic variation. There was a tendency for the response traits to show more genetic variation than the prior traits, which is in agreement with findings in bull calves subjected to a GRF stimulated growth hormone release test (Løvendahl *et al.* 1994). The response of ACTH to CRF stimulation was dependent on the prior ACTH concentration. A similar dependency was found for GH response to GRF stimulation (Løvendahl *et al.*, 1994). The rather low repeatabilities for unstimulated cortisol concentrations are likely a reflection of the random secretory pattern of this hormone, and confirm the results of Hopster *et al*. (1999) of low repeatability of plasma cortisol in cows subjected to repeated venipuncture. The dose of CRF was chosen to give a low to intermediate size of response in ACTH and cortisol, comparable to that expected from an emotional stimulation. This was achieved. Furthermore, the HPA response was of a size similar to that obtained in bull calves following *i.v.* stimulation with CRF at the same dose (Munksgaard *et al*. 1999). Handling was by itself insufficient to stimulate a clear HPA reaction in cows. Therefore, the response to the SI test could not be attributed to responses to handling. Further analyses of these data on genetic correlation will await completion of the experiment and collection of data from more animals, which is needed to obtain lower standard errors on the estimated genetic parameters.
In conclusion, results of this study suggest that genetic variation exists in the responses of the HPA-axis to isolation and CRF-challenge in dairy cattle, and that the heritabilities are moderate.

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