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Adrenal responsiveness to a low-dose ACTH challenge in early and late lactating dairy cows

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ABSTRACT - To improve the evaluation of the chronic stress conditions, the adrenal responsiveness to low dose ACTH stimulation, in different lactation stages, was checked in 56 multiparous dairy cows from 2 herds (25-350 days in milk). Cows were retrospectively ranked in 3 stages: early (<75) average (75-150) and late (>150 DIM) lactation. Herd B (vs. herd A) showed higher basal cortisol and frequency of inflammation. Early stage (vs. others) showed higher basal cortisol, bilirubin, ceruloplasmin and haptoglobin, as well as lower ones of cholesterol and lower rise of plasma cortisol during ACTH challenge (P<0.001). Cortisol peak was also correlated negatively with ceruloplasmin, bilirubin, ROM, and positively with cholesterol, vitamin A and E. Both, basal cortisol and cortisol response to ACTH, are associated to inflammation but in opposite way: basal cortisol positively and cortisol response negatively. This latter results are likely due to lower transcortin synthesis, that could be ensued in early lactating cows suffering inflammation.

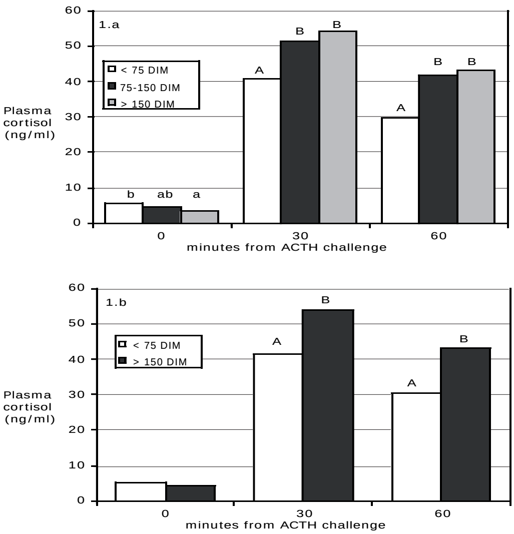
Key words: Dairy cows, ACTH challenge, Stress assessment, Lactation stage.

Introduction – Several hormones (e.g. ACTH, β endorphins, glucocorticoids, etc.) are involved in the stress response. The adrenal gland plays a key-role in hormonal reactions to stress, with a marked increase in glucocorticoids, like cortisol and/or catecholamine; the measurement of plasma cortisol is however more frequently used to study the stress response (Sapolsky *et al.,* 2000). The proper basal level of cortisol is difficult to be measured, because affected by several factors; in particular by the time between capture operations and blood sampling, if higher than 5-10 minutes (from beginning of capture operation to last blood sampling). Furthermore, there is a wide assumption that chronic stress results in a hyper-reactivity of the adrenal cortex. In fact, the animal's response to an acute stressor – such as transport (Broom, 1988) or restraint (Trevisi *et al.,* 2005) – would be greater if the animal had been for long time exposed to other stressors at the farm. This justifies the use of blood cortisol response to exogenous adrenocorticotrophic hormone administration (ACTH challenge), as method to evaluate chronic stress condition. Nevertheless, some cautions are needed regarding the standardization of this method e.g. dosage, distance of time from barn operations, number of blood samples. In addition, physiological factors, as stage of lactation, could also modify the adrenal activity, as previously signalled by Shayanfar et al. (1975). Moreover, the suggestion of Ndibualonji *et al.* (1995) to reduce ACTH dosage, to have a better physiological response and an irrelevant effect on milk yield, was not applied. Aim of the study was a better evaluation of chronic stress conditions of dairy cows, by the measurement of adrenal responsiveness to low doses of ACTH in different stages of lactation.

Material and methods – The research was carried out in two herds of North-East Italy, and concerned 56 multiparous dairy cows after 25 days in milk (DIM), 26 from herd A and 30 from herd B. In the herd A cows were kept in a tie-stall equipped with auto feeder and received the forages twice a day; in the herd B, cows were kept in loose-housing stalls and received a total mixed ration once a day. Twenty five out of 56 animals (7 from herd A and 18 from herd B) were treated in 2 stages: early (<75 DIM) and average-late (>75 DIM) lactation. The others were treated only one time during the lactation (25-35 0DIM). Almost 3 hours after morning meal and far from any other worker operations, cows were captured, using any care to avoid fright, immediately bled from jugular vein and i.v. treated with 20 µg (=2IU) of a synthetic analogue of ACTH ($ACTH_{1\text{-}24}$, Synacthen® – Novartis Pharma AG - Stein, CH). Blood samples were also taken 30 and 60 minutes after ACTH injection, leaving cows restrained in the rack. All the blood samples were collected in vacuum Li-heparin tubes, and immediately stored in iced water. After the blood sampling, the body condition score was evaluated and the presence of injury or clinical diseases, the parity, calving data and milk yield of the last two milkings were recorded. In the blood samples, packed cell volume (PCV) was determined and, after centrifugation (3500g for 16min. at 6°C), plasma cortisol was measured by RIA method (Coat-A-Count; DPC, Los Angeles, CA, USA). Only the samples taken before challenge were also analysed for metabolic profile according to Bertoni *et al.* (2008). Reactive oxygen metabolites (ROM), vitamin A and E were also analyzed, but only in herd B.

The integrated response of cortisol over 60 min were evaluated as area under the curve. For the statistical evaluation, data were subjected to ANOVA using GLM procedure (SAS Inst. Inc., Cary, NC, release 9.1) including in the model herd and stage of lactation (<75, 75-150, >150 DIM) as main factors. Cows treated in two stages of lactation were also subjected to ANOVA using the REPEATED statement in the MIXED procedure of SAS. Finally, Pearson correlations were calculated considering overall data and within stage of lactation.

Figure 1. Plasma cortisol level (ng/ml) during ACTH challenge in all treated cows (1.a), ranked in 3 stages of lactation (<75; 75-150; >150 DIM), or in subjects (1.b) where challenge has been repeated on the same cows in two phases (<75 and >150 DIM). ^{a,b}= P<0.05; ^{A,B}= P<0.01.



Results and conclusions – The herd A, vs. herd B, showed higher milk yield (38.6 vs. 35.5; kg/d P<0.001). As to the blood, some parameter differences were already observed before ACTH challenge: lower levels of cortisol (3.6 vs. 5.3; ng/ml P<0.05) and ceruloplasmin (2.6 vs. 3.4; µmol/l P<0.01), but higher levels of PCV (0.31 vs. 0.29 l/l; P<0.01), glucose (4.0 vs. 3.8 mmol/l; P<0.01), cholesterol (6.7 vs. 4.5 mmol/l; P<0.01) and albumin (37.8 vs. 36.1 g/l; P<0.05). These differences cannot be easily justified; in fact, diet and management were adequate in both herds. Nevertheless, from some of the above mentioned parameters it can be suggested a higher frequency of inflammatory conditions in herd B; according to Bertoni *et al.* (2008), these latter could be explained by the higher ceruloplasmin levels and the lower cholesterol and albumin levels in this herd. Contrariwise no differences between two herds were showed in cortisol response after ACTH challenge. The peak of cortisol level was observed 30 minutes after treatment in both herds, and the average value of plasma cortisol at 30 and 60 minutes after treatment was 47.9 and 36.7 ng/ml respectively. Afterwards, cows were retrospectively ranked in 3 groups according to their DIM: early (<75 DIM), average (75-150 DIM) and late (>150 DIM) lactation. Early, in comparison to average and late groups, showed higher levels of basal cortisol (P<0.05 vs. late group only, Figure 1.a). On the contrary, early group showed a lower rise of plasma cortisol during ACTH challenge (P<0.001) respect to the other groups (figure 1.a). The integrated response of cortisol over 60 minutes measured as area under the curve was consequently lower (P<0.001) in cows in early stage of lactation 1747 vs. 2229 and 2322 ng/ml x 60 minutes for ear-

Table 1. Differences in blood plasma obtained before ACTH challenge in cows ranked in 3 stages of lactation: early (<75DIM) average (75-150DIM) and late (>150DIM). ^{a,b}=P<0.05; ^{A,B}=*P*<0.01.

		Stage of lactation			S.E.
		Early	Average	Late	
Total bilirubin		1.50^{B}	0.81^{A}	0.84^{A}	0.1108
Cholesterol	mmol/l	4.92^{A}	5.96^{B}	6.14^{B}	0.2123
Ceruloplasmin	µmol/l	3.18^{b}	3.01^{ab}	2.80^{a}	0.1039
Haptoglobin	g/l	0.221^{A}	0.104^{B}	0.094^{B}	0.0303

ly, average and late groups respectively. Similar results were observed considering only the cows treated in 2 stages of lactation: <75 and >75 DIM (figure 1.b). Besides some obvious milk yield differences (39; 34; 35 kg/d in early, average and late groups respectively), the cows in early stage of lactation showed lower levels of cholesterol and higher levels of total bilirubin, ceruloplasmin and haptoglobin (Table 1) suggesting again a relationship with inflammation. In the attempt to evaluate the factors responsible of the different cortisol response between early and average-late lactation, correlations with cortisol (basal and raise after challenge) were estimated. The basal cortisol level appeared negatively correlated with DIM (r=-0.41, P<0.001), PCV (r=-0.25, P<0.05), cholesterol (r=-0.30, P<0.01), GGT (r=-0.26, P<0.05), and positively correlated with ceruloplasmin (r=0.27, P<0.01) and total bilirubin (r=0.31 P<0.001). Significant correlations – but with reverse sign - were also observed between cortisol response after ACTH challenge and checked parameters. In particular, the peak cortisol correlations were negative with ceruloplasmin (r=-0.24, P<0.05), total bilirubin (r=-0.35, P<0.001), ROM (r=-0.32, P<0.05) and positive with DIM (r=0.48, P<0.001), glucose (r=0.24, P<0.05), PCV (r=0.25, P<0.05), cholesterol (r=0.35, P<0.001), vitamin A (r=38, P<0.01) and E (r=0.52, P<0.001). Therefore, it seems that both, basal cortisol level and cortisol response to ACTH, are correlated to inflammatory phenomena, but not the same way. Higher basal cortisol in inflamed cows could mean a higher adrenal reactivity, then a chronic stress condition, as suggested by Sapolsky *et al.* (2000). On the contrary, the cortisol response to ACTH challenge is lower during inflammation (e.g. negatively correlated with parameters which are risen by these phenomena and positively with the others: cholesterol, vitamin A and E), suggesting that inflamed cows would be in better welfare conditions (Broom, 1988). Because it is obvious that severe inflammation means on the contrary a welfare depression, the more likely hypothesis of this discrepancy could be: a) the lower doses of ACTH we have utilized are still too high and cortisol level reaches always the maximum; b) the maximum level of cortisol is also influenced by circulating transcortin which in turn is reduced – as other liver synthesized proteins (Greenspan *et al.,* 1986) – in case of inflammation. To conclude the proper evaluation of adrenal response needs an ACTH dose which causes a cortisol peak not affected by transcortin levels.

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