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The ACTH challenge test to evaluate the individual welfare condition

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Abstract - The ACTH challenge test, despite some different opinions, could be a tool to diagnose stress conditions in the animals. A reason of the disagreements could be the procedure, therefore we have studied the effects of ACTH dosage and of the sampling schedule on the cortisol response and interpretation. Two groups of 4 lactating cows, well trained to blood sampling, were alternatively challenged with a high dose (1000 mcg) or with low doses (20, 40 and 80 mcg) of $ACTH_{1-24}$ (Synacthen). Only the very low dose (20 mcg) was also repeated 3 times at 30' intervals. Finally, an ACTH challenge (20 mcg) was carried out in 2 herds with high or low basal cortisol. Blood samples were taken in the following 30' to 180' after i.v. injection.

The highest or the lowest doses induce very similar increase rates and maximum levels of cortisol, if the peak is considered; otherwise higher doses are responsible of more prolonged high values. Furthermore, each cow has a different response that seems unaffected by the basal cortisol level and by the effect of bleeding stress; in fact, repeated low doses of ACTH have only prolonged the cortisol response. It can be therefore concluded that very low doses of ACTH, as 20 or less mcg, can be utilized for ACTH challenge with a bleeding schedule at 30' and 90-120'.

From our results it can be supposed that – with the present ACTH doses - the basal values are less important and that the responsiveness could be evaluated according to the declining rate of cortisol after peak. However ACTH challenge need a further effort for a better standardization.

Key words: plasma cortisol, ACTH challenge, chronic stress, dairy cows.

Introduction

It is well known that cortisol rise is a very common effect of aversive situations and well known is also the physiological mechanism (Sapolsky, 1992). The increase of blood cortisol is part of the stress response and according to Raynaert et al. (1976) it improves fitness by energy mobilization. Corticosteroids has not a protective effect against the stimulus itself, but a broad role in homeostasis protecting the body from overshoot of normal defence (Smith and Dobson, 2002). However, severe chronic stress (prolonged periods of high cortisol concentrations) may decrease individual fitness by immunosuppresion and atrophy of tissues (Munck et al., 1984). In addition, the reproductive success of the animal is decreased (Dobson and Smith, 1995).

An increase in hypothalamic pituitary-adrenocortical activity, causing the rise of blood cortisol, indicates a physiological response to different stressors; consequently a measurement of plasma cortisol is frequently used to study stress response (Sapolsky et al., 2000). Nevertheless the utilization of cortisol as an indicator of stress requires some caution for some type of stress, for the effect of circadian rhythms (Möstl and Palme, 2002) as well as blood sampling itself that can cause stress effects (Negrão et al., 2004). Furthermore, plasma levels of glucocorticoids may also change in response to non-aversive events; for example, corticosteroid levels have been shown to increase during coitus and also while nursing (Manteca, 1998). Finally, it has been suggested that the extent of corticosteroid raise may be more related to the capacity of the animal to learn about the situation than to the real aversion to it (Rushen, 1986); in fact the stress input can decline very much during a prolonged stress situation due to habituation (Smith and Dobson, 2002). The previous statement has been recently confirmed in cattle more or less trained to capture for bleeding, namely untrained animals had a 3-4 times higher cortisol level after 30 minutes (Bertoni et al., 2005).

There is a wide assumption that chronic stress results in a hyper-reactivity of the adrenal cortex, so that the animal's response to an acute stressor – such as transport, for example – would be greater if the animal had been repeatedly exposed to other stressors at the farm (Broom, 1988). This justify the use of blood cortisol level and cortisol response to adrenocorticotrophic hormone (ACTH) administration as indicators of prolonged physical and psychological stress in animals, and therefore to investigate environment, housing, social relationships and management practices stressors on the performance of ruminants. Nevertheless, as reviewed by Manteca (1998), the evidence supporting this assumptions is far from conclusive, and there are papers reporting an increase in adrenal responsiveness following chronic stress, but also papers that report a decrease or no change.

The cortisol responses to ACTH administration is recognized as a method for evaluating adrenal cortex function (Verkerk et al., 1994); nevertheless there are many ways to carry out it (dose rate, peak of cortisol, its rise etc.) and also many ways to justify the adrenal cortex sensitivity. According to some authors reviewed by Hasegawa et al. (1997) the adrenocortical response to ACTH was modified by milk yield, age, ambient temperature and stage of lactation; otherwise Weiss et al. (2004) suggest that in the last 30 years the adrenal cortex sensitivity was justified by genetical or environmental effects. These last authors also suggest that chronic stress rises the adrenal cortex sensitivity only in pigs (or monogastric), while the same situation is characterized by a lower sensitivity in cattle, but the reason is unclear.

The possible reasons of these disagreements could be the followings:

- the chronic stress is not an obvious definition (von Borrel, 2001) and in some experiments the assumption of a chronic stress is attributed to a situation that is pre-conceived to be poor (Lay and Wilson, 2001);
- the difference between cortisol peak and pre-ACTH basal level could be a misleading way of evaluation because the peak values with different ACTH doses (simulating more or less strong stress factors) are similar and not affected by the basal values (Verkerk et al., 1994). Therefore, because the basal value could be higher in low welfare farms, as suggested by Lexer et al. (2004) and Trevisi et al. (2005), the final difference of cortisol change will appear lower.

However this last statement suggests that the ACTH challenge need a better standardization: i.e. nobody has utilized the suggestions to reduce the ACTH to have a better physiological response (Verkerk et al., 1994). Aim of this trial was an attempt to evaluate the effect of ACTH challenge schedule on the response and its interpretation with concern to the adrenal cortex sensitivity.

Materials and methods

The research was carried out with 3 different trials and using only multiparous lactating cows. Cows were treated with a single or repeated i.v. injections of a synthetic analogue of ACTH ($ACTH_{1-24}$ or tetracosactide, Synachten - Novartis Pharma AG - Stein, CH), at different doses. The first 2 trials were carried out in an experimental tied stall barn and the treatments were done about one hour after morning meal and 3-4 hours after milking and other usual management operations.

Trial I: dose effect. Four mid lactating dairy cows were alternatively treated with 4 different doses of ACTH 1-24 (20, 40, 80, 1000 mcg); the interval between every treatment was one week. Blood sample from jugular vein was collected immediately before injection and after 15, 30, 45, 60, 90, 120, 180 minutes.

Trial II: repeated injections. Four mid lactating dairy cows received repeated injections of $ACTH_{1-24}$ at the lowest dose tested in trial I (20 mcg). The treatments were repeated 3 times with 30 minutes interval (0', 30', 60'), while blood samples were taken at the same intervals of first trial.

Trial III: field trial. An ACTH challenge was carried out in 2 different herds, characterized by high or low basal level of plasma cortisol. The herd with high plasma basal cortisol level had a bedded pack and cow were fed with Total Mixed Ratio. The second herd presented a tie-stall equipped with auto feeder and cows received the forages twice a day. About 3 hours after feed distribution and far from any other operations, several multiparous dairy cows (10 and 8 respectively for herd with high or low basal level of plasma cortisol) were captured, using any care to avoid fright, immediately bled from jugular vein and i.v. treated with 20 mcg of $ACTH_{1-24}$. Bleeding was repeated 30 and 45 minutes later,

leaving cows of 1st herd restrained in the rack. After blood sampling, the body condition score (ADAS, 1986) and the presence of injury or diseases were evaluated in each cow, and parity, days in milk (DIM) and milk yield were also recorded.

All the blood samples of the three trials were collected in vacuum Li-heparin tubes and immediately stored in iced water. A small aliquot of each sample was used to determine packed cell volume (PCV), while the remaining portion was centrifuged (3500 g per 16 min. at 5°C) and the plasma was divided in aliquots, stored at –20 °C until required for analysis.

Plasma cortisol was measured by RIA method using a commercial kit (Coat-A-Count; DPC, Los Angeles, CA, USA). The coefficients of variation within and between assays ranged from 3 to 10%. The minimum detectable concentration was 1.4 ng/ml.

For the statistical evaluation of trial I the integrated cortisol response over 180 min was calculated and areas under the curves (AUC), time to peak, peak plasma cortisol levels and 120 minutes concentrations were subjected to analysis of variance for effect of dose and cow (ANOVA; SAS v. 8). The relationship of pre-treatment (0 min) to peak cortisol levels was evaluated by simple correlation analysis (proc. CORR, SAS v. 8). Paired Student's *t*-test was used to compare plasma cortisol concentrations 30 min after each repeated dose and the first (trial II). For field trial, data were subjected to analysis of variance with herd, group and herd-group interaction as main factors (proc. GLM, SAS v. 8)

Results

The results of the first trial, with different $ACTH_{1-24}$ doses, are shown in fig. 1 where it clearly appears that very different doses, 20 mcg vs. 1000 mcg, give very similar peaks of cortisol (no significant difference can be observed). On the contrary, what differentiates the doses is the length of the effect, more prolonged in relation with the higher doses; this is confirmed by the comparison of AUC and cortisol levels at 120' from injection: all data are significantly different ($P<0.01$ at least). Another small difference concerns the time to peak which is significantly shorter ($P<0.05$) only between the lowest (20 mcg) and the highest (1000 mcg) dose.

Furthermore, the average response to 4 doses has shown a significant difference for individual cows ($P<0.05$) when AUC and peaks are evaluated; on the contrary the individual differences are not significant at 120 minutes.

As concerns the influence of pre-treatment cortisol concentration, there is no significant relationship between basal and peak level.

The second trial is concerning the effect of the same low dose (20 mcg) given once or repeated every 30' for a total of 3 doses. The results are shown in fig. 2 in which it appears that a new dose after 30', when cortisol is very close to the peak with 20 mcg of $ACTH_{1-24}$, simply prolong the time of maximum cortisol level, but values are not significantly different; the same occurs with the 3rd dose.

The 3rd trial is aimed to justify the different level of maximum cortisol response to a low dose (20 mcg); in fig. 3 and 4 the pattern of changes of cortisol of cows owned by farm with low or high basal levels of the hormone are shown, which are significantly different ($P<0.001$). In both farms, the cows are retrospectively grouped according to the peak value: <50, 50-60 and >60 ng/ml. The average values at the peak (after 30') are all significantly different ($P<0.05$ at least). Furthermore the number of cows per group is similar: 3, 3 and 2 for the low basal cortisol farm, 3, 4 and 3 for the high cortisol farm, respectively for group <50, 50-60 and >60 ng/ml of maximum cortisol level. The parity, the lactation stage, the milk yield and BCS were slightly different for the 3 groups of the 2 farms, but no significant effect is evident.

Discussion

First of all we have clearly confirmed the results of Verkerk et al. (1994) that very low or relatively high doses of $ACTH_{1-24}$ determine almost the same peak level of cortisol. Nevertheless, the high doses determine a prolongation of the maximum level (fig. 1), which can be also obtained with repeated low doses (fig. 2). According to Crowley et al. (1991) low doses facilitate the release of cortisol, while high doses induce more enzymes for cortisol synthesis. We have some doubts about the

hypothesis as discussed later on, but what appears clearly is that low repeated doses (3 times of 20 mcg every 30' in our case) can maintain the same blood levels of cortisol than 1 mg, at least for 120-150'.

A second result is that the maximum response of cortisol is not influenced by the basal level (fig. 3 and 4); the maximum level does not seem influenced by the welfare conditions too. Therefore, these results do not agree with those obtained by Weiss et al. (2004) or by Trevisi et al. (2005) in naturally challenged cows. Nevertheless the above authors – but not our trials – have not obtained the maximum values or have a more prolonged bleeding time; in both cases it can be supposed that high responder cows show a relatively higher level of cortisol or a more prolonged time with high cortisol (larger area under the curve).

However it is noteworthy the fact that low doses of ACTH are responsible of maximum levels, although they seem different according to some individual traits of the animal (in trial 1 each cow has responded in a similar manner with different doses). We are not able to find a proper justification as suggested by Hasegawa et al. (1997) and Weiss et al. (2004), but it could depends on the different cortisol binding capacity (Negrão et al., 2004); the excess of free cortisol would be quickly conjugated into the liver and excreted via urine and faeces (Möstl and Palme, 2002). If this is true, and we do not have other explanation for the similar maximum values with so different ACTH doses, the prolonged effect of high doses could be perhaps consequence of higher enzyme production, as suggested by Crowley et al. (1991); nevertheless the very close result with low repeated doses demonstrates that adrenal cortex is always able to respond when stimulated (there is no exhaustion). In our case it was true in a short time, but this also occurred in the transported animals; their blood cortisol declined during transportation, but it was suddenly raised at the end of transport when animals were unloaded (Smith and Dobson, 2002). It could be suggested that animals tend to get accustomed to the same stress stimulus (transportation) with a reduction of cortisol, but adrenal can still respond to a new stimulus (unloading).

Conclusions

The ACTH challenge is not yet sufficiently standardized (dose, times of bleeding, response measurement) to allow a proper evaluation of the adrenal responsiveness and its relationship with the animal welfare conditions. For the required future research work, besides a further reduction of dose, a bleeding schedule with few samples (to reduce costs and interfering stressors) and for longer time (90-120') would be attempted. With regard to the interpretation, there are two main possibilities: if the peak is lower than maximum (perhaps 35-40 ng/ml of cortisol) the reached level would be index of responsiveness; if the peak reach the maximum, the responsiveness would be evaluated according to the declining rate of cortisol after the peak. In the first situation the true basal value would be important to measure the real increase of cortisol; in the second one it can be supposed that the basal value could be less important. However, for a better standardization, the ACTH injected dose would be related to the cow weight (metabolic or absolute).

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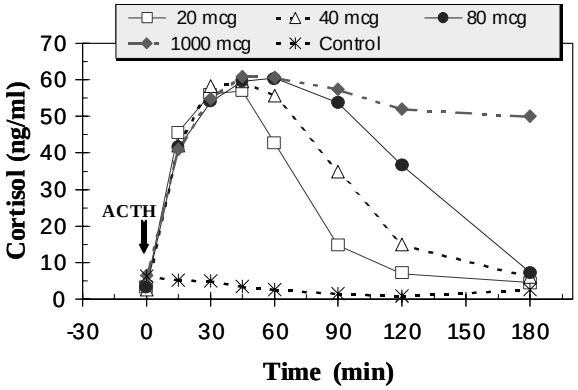

Figure 1 - Mean cortisol response curve after $ACTH_{1-24}$ challenge at 20, 40, 80 and 1000 mcg dose.

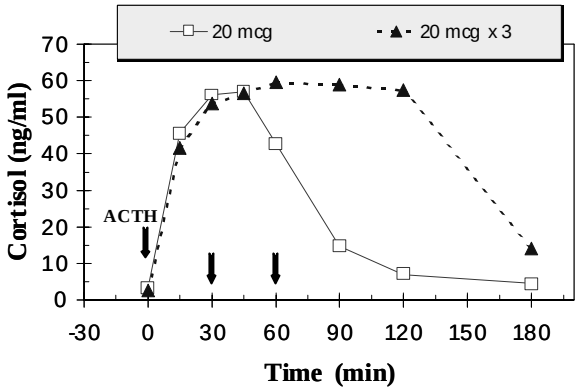

Figure 2 - Plasma cortisol concentrations following the administration of either 20 mcg $ACTH_{1-24}$ at time 0 or the same dose repeated at times 0, 30 and 60 minutes.

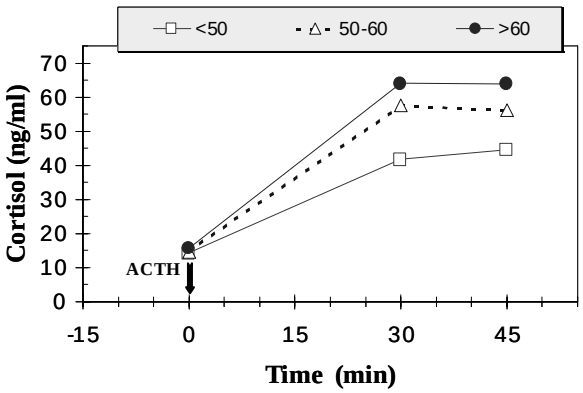

Figure 3 - Cortisol response during ACTH challenge in a herd with high basal levels of plasma cortisol; the cows were grouped according to their adrenal cortex sensitivity (peak value).

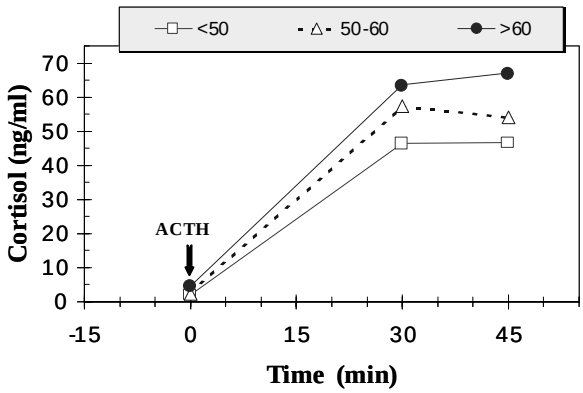

Figure 4 - Cortisol response during ACTH challenge in a herd with low basal levels of plasma cortisol; the cows were grouped according to their adrenal cortex sensitivity (peak value).