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Plasma cortisol variations in dairy cows after some usual or unusual manipulations

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RIASSUNTO – Variazioni del cortisolo ematico in bovine da latte a seguito di varie operazioni di stalla. *Con lo scopo di meglio chiarire le interferenze sulla cortisolemia dovute alle tecniche di prelievo, alla manipolazione degli animali ed all'interazione uomo/bovina, sono state realizzate 4 prove per valutare l'effetto delle seguenti operazioni: prelievo ematico in bovine abituate o meno alla presenza umana, pulizia, mungitura, pesata e mascalcia. I valori basali di cortisolo hanno evidenziato l'usuale variabilità. Dopo 15 minuti dall'inizio delle operazioni la cortisolemia ha mostrato generalmente un picco. L'aumento è stato maggiore negli animali non avvezzi all'interazione con l'uomo ed in rapporto alla "invasività" delle operazioni; anche tra quelli assuefatti si è peraltro riscontrata una notevole variabilità della risposta. La valutazione della cortisolemia nella bovina da latte richiede grande cautela e una meticolosa standardizzazione metodologica.*

Key words: cortisol, dairy cows, management, stressors.

INTRODUCTION – The increase of blood cortisol is a common consequence of the acute stress; this has generally positive effects, despite not completely understood (Sapolsky *et al.* 2000). However, at least in human beings under chronic stress, the hypothalamic-pituitary-adrenal system – responsible of cortisol release – is deregulated, resulting in pathophysiological changes, which may develop into various types of disorders (Tafet and Bernardini, 2003).

According to this, blood cortisol levels could help to diagnose chronic stress; nevertheless it is not so easy because our understanding of long-term stress is poor (Ladewig, 2000). In fact, animals might adapt to long-acting housing conditions that are presumed to be stressful or might not respond with permanent alterations on the various response systems (von Borell, 2001). This could explain the fact that, according to Broom (1988), an hyper-reactivity to adrenocorticotrophic hormone (ACTH) challenge was considered index of chronic stress, while Weiss *et al.* (2004) consider it true in pigs but not in cattle.

An explanation to these contradictory results could be also related to the management and sampling techniques during the challenge trials and that could induce a blood cortisol raise. This have suggested few researches to better clarify which kind of interference can be due to handling and bleeding techniques as well as to more or less common man/cow interactions.

MATERIALS AND METHODS – The research was carried out with 4 different trials.

Trial I. It was done on lactating multiparous cows in a loose-housing stall. Three hours after total mixed ratio distribution and any other worker operation, 12 cows were captured and bled immediately. Bleeding was repeated 20' and 30' (minutes) later, leaving cows restrained in the rack. This trial was carried out in 2 times (6 cows each), to allow a short lapse between bleeding of the 1st and the last cow (about 3'). In addition, the same schedule of bleedings was performed on 3 heifers, less trained to any manipulation and namely to capture.

The other 3 trials were carried out in an experimental tied stall barn. Cows were trained to routinely manipulations (i.e. cleaning, milking, bleeding, weighing) and presence of foreign people.

Trial II. Thirteen cows were bled after a period (about 6 hours) without any disturbance by entrance of people and immediately before the beginning of afternoon usual management operations (MO). Bleeding was repeated 15' later, immediately before individual milking and 15', 30' and 45' after it. The main MO consisted in the entrance of worker and stimulus of cows to stand up, furthermore in the cleaning of stall. To avoid any effect of previous bleeding on the following, each bleeding time occurred in 6 different days and blood samples were collected within 2' from entrance in the barn.

Trial III. Nine cows were bled before weighing and 15' and 30' after it. The operation of weighing was routinely done every 2 weeks and consisted in tying each cow and leading up the scale in the same barn.

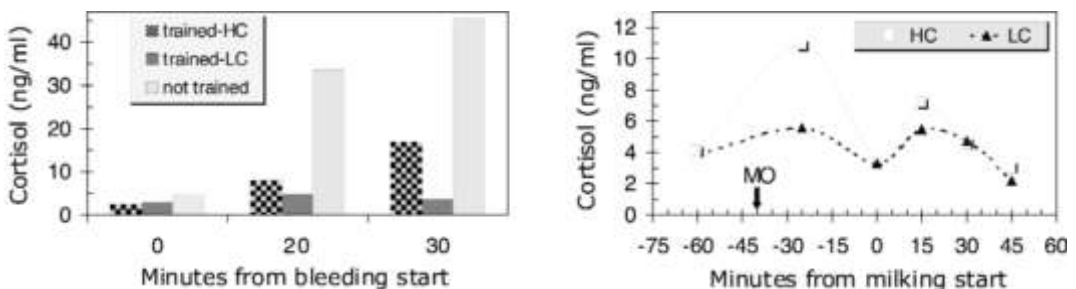
Trial IV. Four cows were moved into a stock box for hoof dressing; they were bled -60', 0', 30', 60', 120', 210' and 300' from the beginning of trimming that lasted 20' for 3 animals and 35' for the 4th one. At the same time, 4 cows were used as control.

All the blood samples were collected in vacuum Li-heparin tubes, immediately stored in iced water until centrifugation (3500 g x 16' at 5°C). The obtained plasma was stored at -20°C until cortisol determination by RIA method using a commercial kit (Coat-a-count, Diagnostic Products Corp., USA). The statistical comparison between means was performed by the Student's t test.

RESULTS AND CONCLUSIONS – *Trial I.* Effects on cortisolemia in cows restrained for 30' and belonging to herds trained or not to human presence and manipulation, have been showed in figure 1 (left). Trained cows were divided in LC (8 cows) and HC (4 cows) groups, according to the raise of cortisol after 1st bleeding: low (<2 folds) or high (>2 folds). LC cows showed a slight cortisol increase after 20' (P<0.08) from the beginning of the restraint; while in HC group it increased more and progressively till 30' (P<0.01). The same behaviour was observed in heifer not trained to manipulations, but the raise was much more marked (about 46 ng/ml and over 11 times *vs.* 1st bleeding).

Trial II. Figure 1 (right) showed changes of plasma cortisol in response to cleaning of stalls and to milking. After both events, cortisol was shortly raised, but the variability was very high. A part 2 cows excluded for the very high values (leg-blockage utilised during milking), the remaining 11 cows were divided in two groups (LC and HC). In LC cows (45% of them) the changes of cortisol were not relevant, although sometimes significant, while HC cows have showed a considerable raise after both events (P<0.05), but more pronounced after MO (P<0.01). Basal values for the two groups were similar. It is therefore confirmed (as suggested by Weiss *et al.*, 2004) that the bleeding operations can represent a small stress for cows very familiar with human manipulations, conversely it became a strong stressor for untrained animals. It is also confirmed that milking can cause stress conditions (Weiss *et al.*, 2004), but almost negligible if it is friendly; on the contrary, the “wake up” induced by cleaning operations represents a stronger stressor.

Figure 1. *Left.* Plasma cortisol changes in response to bleeding in cows trained or not to manipulations. *Right.* Idem to management operations (MO) and milking. HC= raise >2 folds; LC= raise <2 folds

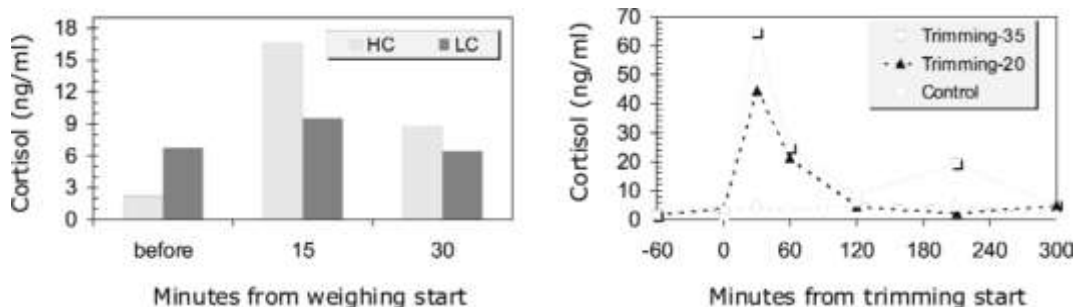


Trial III. The cortisol pattern of change of cows after weighing is showed in figure 2 (left). Cows were again divided in 2 groups (LC, 5 cows and HC, 4 cows) according to the cortisol level observed before and after the beginning of weighing. All cows reached the peak of cortisol 15' after it ($P < 0.001$ only in HC). LC cows had high level of cortisol before weighing (about 3 times *vs.* HC), but also higher variability.

Trial IV. Figure 2 (right) has also showed the effect of trimming hoofs, separately according to the time spent for the operation. In this case the cortisol peak was observed 30' after the beginning of events ($P < 0.001$) and the complete recovery to pre-trimming levels was slower than previous trials (not before 180'). In addition, the increase seemed related to intensity of stress, as suggested by higher peak and longer time to recover pre-trimming levels in the cow with longer trimming.

In the last 2 trials, there was a disruption of the routine: the cows were leaded away from their place to be "close" on the scale or into the stocks. This could justify the stronger effect and completely different level according to duration and strength of the stressors.

Figure 2. *Left.* Plasma cortisol changes in response to weighing operation. *Right.* Idem to trimming for 20 or 35 minutes. HC= raise >2 folds; LC= raise <2 folds



In conclusion, the level of cortisolemia in dairy cows is highly influenced by several and usual manipulations, for this reason schedule of bleedings must take in account these effects and their duration as well as the habit to bleeding. Furthermore, it is noteworthy that, particularly to moderate stressors, cows can be distinguished in high and low responder as showed by Weiss *et al.* (2004); nevertheless, it remains to be better clarified whether this depends on the specific sensitivity or it is only random.

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