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THE EFFECT OF DURATION OF MANUAL RESTRAINT DURING BLOOD SAMPLING ON PLASMA CORTISOL LEVELS IN CALVES.

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Abstract:

Many studies on stress and pain rely, solely or mainly, on plasma cortisol assessment. Confounding factors, such as handling, may cause a release of cortisol making the interpretation of the results difficult. We looked at the influence of duration of restraint on the plasma cortisol levels of one-to-two month old calves. Forty-three calves were divided into four groups according to the interval between restraint and blood sampling: i) Group 0, immediate blood-sampling; ii) Group 0.5M, half a minute restraint; iii) Group 1M, one minute restraint and iv) Group 2M, two minutes restraint. The only increase in plasma cortisol, compared with all the other groups, was seen with blood sampling after two minutes of restraint. This study provides evidence to suggest that cortisol released as a result of handling stress is not evident if blood sampling is carried out within one minute of restraining calves.

Key words: calves; cortisol; handling; stress.

Introduction

Various hormones (eg ACTH, glucocorticoids, catecholamines, prolactin, etc) play a role in the stress response of animals (for a review see Matteri et al 2000). It is well established that glucocorticoid production, following the activation of the hypothalamic-pituitary-adrenal (HPA) axis, is part of an emergency response (Broom & Zanella 2004) intended to defend the organism against stressful

conditions (M.stl & Palme 2002). It is for these reasons that cortisol is medically termed 'the stress hormone'.

The concentration of cortisol in blood is widely used as an indicator of stress, although caution in the interpretation of results is advised because an increase does not occur with every type of stressor (Broom & Johnson 2000) and because a wide variety of stressors can activate the HPA system (Molony & Kent 1997; Broom & Johnson 2000). In farm animals, examples of these are: weaning (Hickey et al 2003); social isolation (review by Cockram 2004); transport (Crookshank et al 1979; Grigor et al 2004); social mixing (Arthington et al 2003); novelty (van Reenen et al 2005); restraint and handling (Ewbank et al 1992) and multiple venipuncture (Hopster et al 1999). See also review by Lane (2006).

Sample collection, which often involves confinement and handling of animals, may be, in itself, stressful and may confound the results of studies (Cook et al 2000). Some authors have used in-dwelling catheters applied some time before the study begins but this implies regular flushing of catheters and may cause discomfort or infection. Also, this method does not preclude all handling. Non-invasive measurements of cortisol (milk, saliva, urine and faeces) may reduce or prevent this disadvantage but only provide information on overall levels and, thus, is more useful for studies on chronic stress. For the main disadvantages of noninvasive measurements of cortisol, see Lane (2006). So, to validate blood cortisol as an indicator of stress or pain caused by a particular procedure, all redundant effects should be eliminated (Cook et al 2000).

Calf blood cortisol is useful for the evaluation of stress and pain after routine farm procedures, such as disbudding, taildocking and castration (Molony et al 1995; Morisse et al 1995; McMeekan et al 1998; Faulkner et al 2000; Schreiner & Ruegg 2002; Sutherland et al 2002; Stafford et al 2003; Stilwell & Lima 2004, 2008). Although all studies use control groups that are not subjected to the procedures, the effect of duration between restraint and blood sampling on blood cortisol levels of young calves should be taken on account. This study was designed, therefore, to measure the effect of time between first restraint and blood sampling, on plasma cortisol levels of young calves.

Table 1 Mean (± SD) blood cortisol levels of calves restrained for differing periods of time.

Time to sampling (Group)	n	Age (days)	Cortisol (nmol l^{-1})
Immediate	10	47 ± 11	11.71 ± 7.97[a]
0.5M	9	46 ± 13	18.39 ± 13.85[a]
1M	10	54 ± 10	16.34 ± 14.33[a]
2M	13	49 ± 11	40.12 ± 31.10[b]

Different superscripts indicate significant differences ($P < 0.05$).

Materials and methods

The study site

This study was carried out at a large cattle rearing unit which receives between 100 and 200 young calves each month from dairy farms. The great majority are Holstein-Friesian, but some crossbreeds are seen (Holstein × Limousin and Holstein × Belgian Blue). Transport distances from farms of origin range from 2 to 200 km and on arrival all calves are put in individual boxes and receive an electrolyte solution.

Animals are fed twice a day with a commercial milk-replacer in individual buckets. Water and concentrate are available all day. New straw is added every three-to-four days, but bed material is only completely removed when the calf is weaned and moved to group paddocks. Calves have close contact with herdspersons at feeding, adding of bedding and during twice daily individual visual monitoring. Weaning occurs at approximately two months of age.

Study animals

Forty-three male Holstein-Friesian calves were included in the study. They were housed in the same building along four rows and, although age varied between 31 and 67 days (Table 1), all calves were milk fed.

The animals underwent systematic allocation to different groups. Starting at one end of the first row every four calves were distributed to the following four groups, according to the time between entering the individual pen and blood sampling: i) Group 0, immediate blood sampling; ii) Group 0.5M, 0.5 min restraint; iii) Group 1M, one minute restraint and iv) Group 2M, two minute restraint. Restraint was carried out by squeezing the calf gently against the pen wall with a knee while holding the head with one hand. This was done by an experienced veterinary surgeon and no excessive force was needed with any of the animals. A second person, five metres from the pen, measured the time and advised when venipuncture and blood sampling should be done. One calf that should have been included in Group 0.5M was excluded because of signs of illness. The last three calves of the last row were included in Group 2M. There were no age differences between groups.

Blood samples (7 ml) were taken into a heparinised tube by left jugular venipuncture. Blood was immediately centrifuged and frozen (–20ºC). Cortisol was assayed in duplicate and measured by a validated solid radioimmunoassay, without extraction, using commercial kits (Coat-A-Count®, Diagnostic Product Corporation, Los Angeles, CA, USA). The inter-assay coefficient of variation for cortisol was 5.5% for the level of 1µg dl^{-1} and 1.9% for the level of 5 µg dl^{-1}.

Statistical analysis

The distributions and variance of the data were shown not to be normally distributed by Levene and Shapiro-Wilks tests. Significant differences between the four groups were then determined by the Mann Whitney *U*-test following a Kruskal-Wallis, one-way analysis of variance. Computer software SPSS version 14.0 was used for the analysis.

Results

The results (Table 1) showed a significant difference between Group 2M and Group 0 ($P = 0.002$), Group 0.5M ($P = 0.03$) and Group 1M ($P = 0.021$). Individual variation in blood cortisol levels was very large within each group but especially in the 2M group. Within each group we also compared cortisol levels of animals that were younger than the mean age with those that were older than the mean age and found no differences (data not shown in table).

Discussion and conclusions

The question, 'how long after an animal has been stressed by handling and restraint will the cortisol response be evident?', has not been answered for young calves used for studies on pain associated with disbudding, dehorning, tail docking and castration.

Hopster *et al* (1999) found that initial collection within one minute of restraint did not alter baseline cortisol in dairy cows, but repeated venipuncture at 15 min intervals caused an increase in cortisol in primiparous cows less accustomed to handling. Our study used young dairy calves that had been accustomed, since birth, to human proximity and contact. Although restraint was easy and the animals did not show any evidence of distress, we did show that handling alone does cause a significant cortisol response, even in very young calves that were used to human contact. However, we also showed that cortisol levels are not affected if blood sampling is done immediately after restraint (up to one minute of restraint, at least). This suggests that when studies on distress and pain in calves are carried out, non-treated control groups may give reliable information on baseline plasma cortisol levels, providing that blood sampling is carried out by an experienced operator and takes place within one minute of first handling and restraint.

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