

Matricaria chamomilla CH₁₂ decreases handling stress in Nelore calves

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Matricaria chamomilla CH₁₂ is a phytotherapeutic or homeopathic product, which has been used to reduce stress. Here, we examined its effect on preventing handling stress in bovines. Sixty Nelore calves were randomly distributed into two equal groups. One group was administered *Matricaria chamomilla* CH₁₂ in diet and the other the 'control' was not. Animals in both groups were maintained unstressed for 30 days to adjust to the feeding system and pasture, and were then stressed by constraint on the 31th, 38th, 45th and 60th experimental days. Blood samples were taken on these days after animals had been immobilization in a trunk contention for 5 min. Stress was followed by analyzing serum cortisol levels. These peaked on the 45th day and then decreased, but not to baseline, on the 60th day. On the 45th day cortisol levels were significantly lower in animals fed *Matricaria chamomilla* CH₁₂, suggesting that this product reduces stress. These effects may be a consequence of its inhibiting cortisol production and its calming and anxiolytic effects.

Key words: bovine, cortisol, handling, *Matricaria chamomilla*, Nelore calf, stress

Introduction

Neuroendocrine stress responses are regulated by the hypothalamic-pituitary-adrenal axis (HPA), which promotes plasmatic cortisol release [38,26]. This response is vital to an organism that has to cope with a challenging environment [19].

Bovine stressors may be either physical or psychological. The most referenced stressors are vaccination [30], immobilization

[19], handling in a corral [37], installation [5], fear [28], presence of unfamiliar people [23], transportation [7], food and water deprivation [37], hot or cold environment [5], cirurgical procedures [6], crowding [7], isolation [9], confinement [11] and natural physiological changes (parturition, lactation, weaning) [37].

The effects of stress on bovine economy and production are remarkable. For example, in Argentina it has been estimated to account for losses of 50 million dollars per year [7]. Moreover, the effects of the above stressors are increased when bovines are held in modern regimes, such as, intensive systems that impose severe stress [37] and require higher investments [11]. Under such conditions, stress impairs cattle growth, weight gains, milk production, and meat quality [11,10,26,37].

Phytotherapy or homeopathic products, such as, the chamomile *Matricaria chamomilla*, have been used to decrease stress. Moreover, apigenin the active principle of chamomile, decreases cortisol plasma concentrations [41] and has sedative [39], analgesic [3], anxiolytic [39], anti-inflammatory [33] and immunomodulatory effects [1].

Although the phytotherapeutic effects of chamomile are well documented, its anti-stress effect on bovines is not as well known. Thus, in the present study, we evaluated whether the chamomile-based product, *Matricaria chamomilla* CH₁₂, prevents or reduces handling stress response in Nelore bovines.

Materials and Methods

Animals

Sixty Nelore calves (*Bos indicus*), about 12 months old, belonging to a farm situated in Lutecia, SP, Brazil, were studied. Animals were fed *Brachiaria decumbens* from an extensive pasture system and supplemented with commercial mineral salt in an *ad libitum* manner.

The experiment was carried out during the spring (September

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to October) of 2003 in a tropical area, which is characterized by a rainy season from October to April and a dry season from May to September, with an annual precipitation of about 1300 mm, a relative humidity of approximately 64%, a mean temperature 25°C, and an altitude of 602 m. Nelore cattle adapt well to these conditions.

Matricaria chamomilla CH₁₂

Matricaria chamomilla CH₁₂ (Fator Estresse; Arenales Fauna & Flora, Brazil) is composed of *Matricaria chamomilla* CH₁₂, milk CH₁₂, *Bixa orellana* (0.75 g) and sucrose (100 g).

Matricaria chamomilla CH₁₂ administration

The animals were randomly distributed into two groups (30 animals/group): one received feed with mineral salt supplemented with *Matricaria chamomilla* CH₁₂ and the other group without this supplement (control). In both groups the animals were maintained unstressed for 30 days to adjust to the feeding system and adaptation to pasture, and were then stressed on the 31th, 38th, 45th, and 60th days of the experiment. Each animal in the *Matricaria chamomilla* CH₁₂ group ingested about 2 g of *Matricaria chamomilla* CH₁₂ daily. The quantity of this supplement consumed by the animals was estimated by incorporating *Matricaria chamomilla* CH₁₂ into mineral salt every day and calculating the mean individual salt consumption (daily difference between salt weight offered and amount left after 24 h divided by the number of animals).

Blood samples were taken on days 31th, 38th, 45th, and 60th after immobilization, which was performed by leading animals to a corral in the morning of the sampling day, and restraining each animal in a trunk contention for 5 min immobilization blood samples were taken (10 ml) from the jugular vein and stored in vacuum tubes not containing anticoagulant. These tubes were kept in an insulated container in ice and soon centrifuged 2,500 rpm by 10 min. Serum samples were stored at -20°C for subsequent serum cortisol determination.

Stressors imposed on cattle

The stressors imposed on calves are: handling pen, presence of unfamiliar people during handling, immobilization in a trunk contention for 5 min and harvesting blood sampling.

Serum cortisol determination

Serum cortisol levels were determined using a commercial solid-phase radioimmunoassay kit (Diagnostic Products, USA) and counted using an Auto-Gamma Count Cobra II (Packard Bio Sciences, USA).

Statistical analysis

Data were normalized by $[(x + 0.5)^{1/2}]$ and significant effects were detected by repeated measures ANOVA and

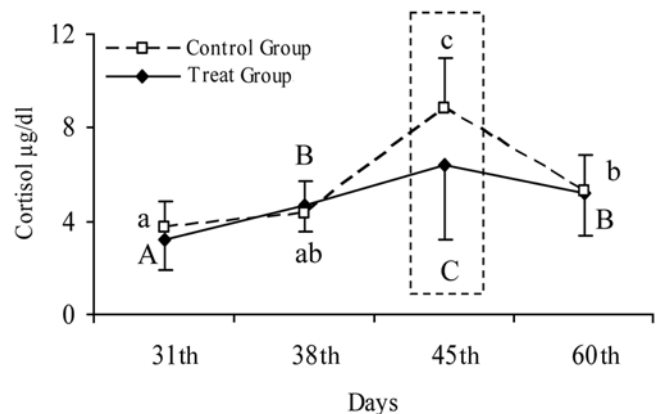


Fig. 1. Effect of *Matricaria Chamomilla* CH₁₂ on calf serum cortisol. Mean cortisol levels (\pm SD) of treated and control groups (30 animals/group). Means with the dotted box are statistically different from each other ($p < 0.05$). Means with at least common lower case letter are similar within the given group.

then compared using the Tukey test. The error probability was set at 5% [4].

Results

Animals in both groups showed increased serum cortisol from the beginning of the experiment and peak values were reached on the 45th day. Cortisol levels then decreased gradually but did not reach basal values on the 60th day (Fig. 1). Highest serum cortisol levels were observed in both groups on day 45, but this was significantly lower in animals treated with *Matricaria chamomilla* CH₁₂ (ANOVA, $F = 8.54$; $p < 0.01$).

Discussion

The handling procedures imposed on cattle in holding pens are stressful, and increase serum cortisol levels, although these levels are almost completely reestablished after animals have adjusted. Cattle stress is attenuated by the chamomile-based product *Matricaria chamomilla* CH₁₂, possibly because this material reduces serum levels of adrenocorticotrophic hormone (ACTH) and has sedative, anxiolytic, and miorelaxing properties.

On the 31th experiment day after animals had adjusted to the feeding system and before or after applying constraint stress for 1 h, serum cortisol levels were 3.16 μ g/dl in animals fed *Matricaria chamomilla* CH₁₂ and 3.68 μ g/dl in controls. These values are close to the basal cortisol levels of 3.29 μ g/dl found in Zebu cattle by Aragón *et al.* [2] and by Vásquez and Herrera [37].

In this experiment, many factors may have caused the cortisol increase. In fact, handling a primary stressor in farm animals and is known to impair animal welfare [5,9,21,28]. Moreover, leading cattle to a holding pen is a forced physical

exercise [12,22,34], as is restraint in a 'Brete' chute [19,35]. In addition, blood sampling [28] and the presence of unfamiliar people during handling [9,16,17,23] impose additional stress. The handling pen is a novelty for animals [20,29,38], and shouting and physical aggression by handling [16,32], and fear caused by the conditions mentioned above [5,14,28] also acted as stressors in the present study.

Neuroendocrine stress response consists of the activation of the hypothalamic pituitary axis, which results in the secretion of cortisol by the adrenal gland [8,26,38]. This is a possible explanation the results obtained from the 38th day to the end of the experiment in both the control group and the group treated with *Matricaria chamomilla* CH₁₂. Moreover, although no statistical difference was found, serum cortisol levels were higher than the basal concentrations obtained by Aragón *et al.* [2] and Vásquez and Herrera [37], thus supporting the notion that the animals were slight stressed.

On the 45th day of this experiment, control animals showed cortisol levels 38.4% higher than those of animals treated with chamomile. This response is in agreement with the results reported by Ohno *et al.* [31], who found that *Matricaria chamomilla* reduces cortisol production by about 47.5% in human adreno-cortical H295R cells. In other mammals, the flavonoid apigenin contained in *Matricaria chamomilla* [25,33,40,42] was found to act on the nervous system by diverse and complex mechanisms [27,13].

On the 60th day of the experiment, cortisol levels had dropped off in treated and control animals, and almost reached basal levels. This reduction can be attributed to adjustment to handling procedures [18,28,36]. Adjustments to stressors are caused by changes in the central nervous system at different levels, i.e., effects on hypothalamus and pituitary function; on neurosteroids and neurotransmitters; on the limbic system (mainly the amygdala and hippocampus) and on the hypothalamic pituitary axis [6], which decrease axis response to stressors [19]. The capacity of bovines to adjust to stress differs among individual animals due to genetic [15,19] and temperament features, i.e., docile animals adjust more easily [15].

The results of the present study provide evidence that *Matricaria chamomilla* CH₁₂ decreased stress in bovines. We hypothesize that the mechanisms involved are the inhibition of cortisol production and the calming and anxiolytic effects of this material.

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