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Koncentracija kortizola u slini junica u tovu držanih u obogaćenome okolišu pri različitim gustoćama naseljenosti

**Matković, K., Šimić, R., Barić Rafaj, R., Lolić, M., Ostović, M.**

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**Fakultet agrobiotehničkih znanosti Osijek, Poljoprivredni institut Osijek**

Faculty of Agrobiotechnical Sciences Osijek, Agricultural Institute Osijek

# SALIVARY CORTISOL CONCENTRATION IN FATTENING HEIFERS KEPT IN AN ENRICHED ENVIRONMENT AT DIFFERENT STOCKING DENSITIES

Matković, K.<sup>(1)</sup>, Šimić, R.<sup>(2)</sup>, Barić Rafaj, R.<sup>(1)</sup>, Lolić, M.<sup>(3)</sup>, Ostović, M.<sup>(1)</sup>

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## SUMMARY

*The aim of the study was to assess the effect of environmental enrichment and different stocking densities on the level of stress in the fattening cattle by measuring salivary cortisol concentration. A total of 320 saliva samples obtained from heifers during the final four-month fattening period was analyzed by a colorimetric competitive enzyme immunoassay. The heifers were housed at 3.3 and 4.5 m<sup>2</sup>/animal in standard production conditions that were additionally enriched with a mechanical grooming brush and salt blocks in experimental groups. The study results have demonstrated that a cortisol concentration at the end of fattening was significantly higher, as compared with the initial levels in all heifer groups, without significant intergroup differences. There was no significant correlation between the usage of a grooming brush and cortisol concentration, whereas a significant negative correlation was detected between the salt block usage and cortisol concentration, suggesting a beneficial effect of this simple, commercially available and relatively inexpensive environmental enrichment.*

**Keywords:** cattle, cortisol, stress, enriched environment, stocking density, animal welfare

## INTRODUCTION

Stress is a biological response of an individual activated upon any homeostatic threat (Moberg, 2000; Chrousos and Kino, 2005). Because of its increased secretion in stressful situations, cortisol, a hormone produced by the adrenal cortex, is considered to be an indicator of the animal stress and animal welfare. In normal conditions, an additional energy is thus produced to overcome a stressful, perilous situation (Sjaastad et al., 2010). A cortisol concentration is influenced by numerous environmental factors, including stocking density (Kim et al., 2015), also genetic factors, as well as previous experiences; therefore, strict comparisons between various studies are not recommendable. In cattle, the cortisol levels are highly variable, ranging from 0.5 to 9 ng/mL during a routine handling (Grandin, 1997).

Environmental enrichment is defined as a technique to improve an animal's biological functioning by modify-

ing its environment (Newberry, 1995; Panchbhai and Thakur, 2016). Environmental enrichment assists the animals to cope with various stressors by stimulating the exhibition of their physiological behavioral patterns while reducing their boredom and frustration (Mandel et al., 2016). In the European Union, environmental enrichment is mandatory for poultry and pigs but not for the cattle, although there are recommendations and opinions advocating it. Numerous cattle-oriented studies of an enriched environment demonstrated a variety of benefits (e.g., Ishiwata et al., 2006; DeVries et al., 2007; Newby et al., 2013; Mandel et al., 2016; Ninomiya,

(1) Prof. Dr. Kristina Matković, Prof. Dr. Renata Barić Rafaj, Assoc. Prof. Mario Ostović (mostovic@vef.unizg.hr) – University of Zagreb, Faculty of Veterinary Medicine, Heinzelova 55, 10000 Zagreb, Croatia, (2) Ranka Šimić, Ph. D. – Ministry of Agriculture, Veterinary and Food Safety Directorate, Planinska 2a, 10000 Zagreb, Croatia, (3) Marica Lolić, Ph. D. – Croatian Veterinary Institute, Vinkovci Veterinary Department, Josipa Kozarca 24, 32100 Vinkovci, Croatia

2019), whereas only few studies investigated a level of stress in the fattening cattle (Kim et al., 2015; Park et al., 2020a and 2020b).

The present study aimed to assess the effect of an enriched environment at different stocking densities on the level of stress in fattening cattle while determining a cortisol concentration.

## MATERIAL AND METHODS

The study was approved by the Ethics Committee of the Faculty of Veterinary Medicine, University of Zagreb.

The study was carried out at a commercial cattle farm during a final four-month fattening period (February–May) and involved 66 yearling heifers of various breeds, equally distributed in two experimental and two control groups. The control groups consisted of 19 heifers (3.3 m<sup>2</sup>/animal) and 14 heifers (4.5 m<sup>2</sup>/animal) housed in the standard production conditions. In the experimental groups, these standard conditions were additionally enriched with a mechanical grooming brush (Albert Kerbl GmbH, Germany) and pure salt blocks (Royal ilaç, Turkey) at identical stocking densities. A grooming brush was mounted on the wall in the midst of an enclosure, enabling the animals to groom withers and sides, whereas two salt blocks (10 kg each) were placed along the feeding trough, with no need of replenishment during the study period in both experimental groups. In each group observed, there were two heifers of the Charolais, Belgian Blue, and Hereford breeds, 13 Simmental heifers were present in the groups with the higher stocking densities, while eight Simmental heifers were present in the groups with the lower stocking densities. All animals were kept in the semi-open, deep-littered pens (5.2 m x 12 m), with approximately 3.5 kg of straw provided per day and per animal. Each pen was equipped with two automatic drinking bowls and a 12-m feeding trough. The feed was delivered twice per day (at 7:00 a. m. and at 2:00 p. m.). The feed composition was as follows: super concentrate (8.61%), sodium bicarbonate (0.05%), straw (1.59%), green silage (45.11%), corn grits (29.19%) and beet pulp (15.92%). The super concentrate mixture (*Belje plus d. o. o.*, Croatia) was composed of crude protein (38.10%), crude fat (2.19%), crude fiber (15.07%), crude ash (15.50%), calcium (3.24%), phosphorus (0.74%) and sodium (0.98%), including vitamin A retinyl acetate (33,750 IU), vitamin D3 (4050 IU), vitamin E (135 mg), copper sulfate pentahydrate (37.5 mg) and antioxidant BHT (300 mg), which were added per one kg of mixture.

Saliva samples for cortisol determination were collected subsequent to the matutinal feeding, in the two-week intervals, starting after the second fattening week due to an acclimatization period, eight times until the end of fattening. The sampling was performed in 10 animals per group, each time including all Charolais, Belgian Blue, and Hereford heifers and four randomly selected Simmental heifers. A total of 320 saliva samples was tested. The procedure of saliva sampling took

a few minutes. The animal was taken to a cattle chute within the enclosure with a minimum force applied; then, a 2.5 x 5 x 2.5 cm sponge was inserted into the buccal part of the oral cavity, and saliva was squeezed into the tube through a sterile jet. The saliva samples were processed by a preanalytical centrifugation and stored at -20 °C prior to an analysis by a colorimetric competitive enzyme immunoassay (*Cortisol EIA* kit, Enzo Life Sciences, Switzerland) and a microplate reader at 450 nm (BioTek Instruments, USA).

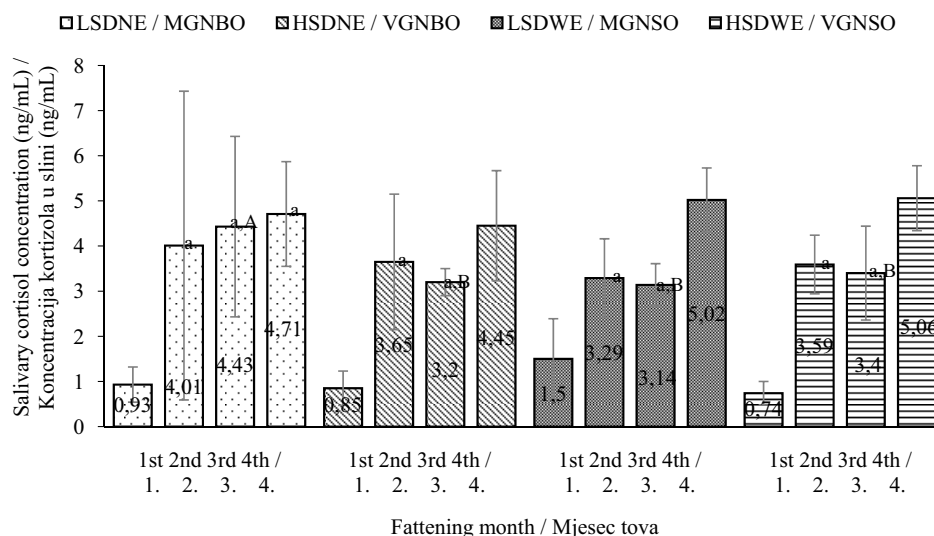
A grooming brush and salt blocks usage was counted by a direct observation of heifers for two hours per week throughout the study period, implying that an animal used a grooming brush for more than five seconds and licked a salt block for more than three seconds. During weekly observations, an animal could have been repeatedly recorded while exhibiting the same or a different behavior. The results pertaining to the grooming brush and salt blocks usage have been reported previously (Matković et al., 2020).

The data were analyzed using the *Statistica* v. 13.5 reference program (TIBCO Software Inc., Palo Alto, CA, USA), *Factorial ANOVA* and a post-hoc LSD test, and the Spearman rank-order correlation. The level of  $P \leq 0.05$  was considered significant in all tests.

## RESULTS AND DISCUSSION

This report describes the effect of an environment enriched with a grooming brush and salt blocks on a salivary cortisol concentration in heifers housed at different stocking densities during the final four months of fattening. As depicted in Figure 1, a cortisol concentration was at the lowest in the first fattening month in all heifer groups, yielding significant differences ( $P < 0.05$ ) in comparison to all other study months. These results suggest the effects of confinement length and heifer age exerted on the cortisol concentration, with the age-related differences being evident due to a physiological state rather than due to the age itself (Vesel et al., 2020).

So, a cortisol concentration was significantly lower ( $P < 0.05$ ) also in the second and in the third month, as compared with the fourth month, i.e., the last fattening month in all groups of heifers, with the exception of a group kept in a non-enriched environment at a lower stocking density ( $P > 0.05$ ) (Figure 1). A fattening month x group interaction was detected ( $P = 0.05$ ), with a significantly higher ( $P < 0.05$ ) cortisol concentration recorded in the third fattening month in the heifer group kept in a non-enriched environment at a lower stocking density, as compared with all other groups. No other significant ( $P > 0.05$ ) intergroup differences in cortisol concentration were recorded (Figure 1). The results obtained could be explained by the more frequent aggressive interactions of heifers in a non-enriched environment also at a lower stocking density (Matković et al., 2020), i.e., with a more room available for their manifestations, thus maintaining the steady cortisol concentrations until the end of fattening in this heifer group (Figure 1).



**Figure 1. A salivary cortisol concentration in heifer groups kept in a non-enriched and enriched environment at different stocking densities during a final four-month fattening. The values are expressed as a mean  $\pm$  standard deviation. LSDNE – low stocking density with no enrichment; HSDNE – high stocking density with no enrichment; LSDWE – low stocking density with enrichment (grooming brush + salt blocks); HSDWE – high stocking density with enrichment (grooming brush + salt blocks). <sup>a</sup> all values within the same group differed significantly ( $P < 0.05$ ), except the marked ones; <sup>A,B</sup> values marked with different letters differed significantly ( $P < 0.05$ )**

*Grafikon 1. Koncentracija kortizola u slini u skupinama junica držanih u neobogaćenome i obogaćenom okolišu pri različitim gustoćama naseljenosti tijekom završnoga četveromjesečnog tova. Vrijednosti su izražene kao aritmetička sredina  $\pm$  standardna devijacija. MGNBO – mala gustoća naseljenosti bez obogaćenja; VGNBO – velika gustoća naseljenosti bez obogaćenja; MGNSO – mala gustoća naseljenosti s obogaćenjem (četka za njegu + blokovi soli); VGNSO – velika gustoća naseljenosti s obogaćenjem (četka za njegu + blokovi soli). <sup>a</sup> sve vrijednosti unutar iste skupine značajno se razlikuju ( $p < 0.05$ ), osim označenih; <sup>A,B</sup> vrijednosti označene različitim slovima značajno se razlikuju ( $p < 0.05$ )*

Grooming brushes are generally used as an environmental enrichment in dairy cows (DeVries et al., 2007; Mandel et al., 2013; Newby et al., 2013; McConnachie et al., 2018), whereas the salt blocks have not been widely used in cattle breeding. Previous studies have proven that the fattening cattle favorably responds to the grooming brush, in particular because its high motivation for grooming is met (Wilson et al., 2002; Ninomiya, 2019; Park et al., 2020b), and it equally favorably responds to the salt blocks because its nutritional requirement and a necessity to explore the environment (Pelley et al., 1995). The studies conducted by Šimić et al. (2018) and Matković et al. (2020), which assessed different stocking densities in addition to an environmental enrichment, demonstrated that the environment enriched with a grooming brush and salt blocks has reduced the aggressive behavior expression in the fattening heifers. The latter study also found a significant positive correlation between a salt block usage and the type of aggressive behavior (displacement), which the authors were inclined to attribute to a high heifers' interest to access the salt blocks. This was also confirmed by the results recorded in the present study. In this study, using a grooming brush had no effect on cortisol concentration ( $r = -0.257$ ;  $P = 0.45$ ), but there was a significant negative correlation between a salt block usage and cortisol concentration ( $r = -0.623$ ;  $P = 0.04$ ).

## CONCLUSION

Accordingly, a cortisol concentration was higher at the end of fattening, as compared to the beginning

in all heifer groups, without any intergroup differences. There was no effect of grooming brush on cortisol concentration; however, providing salt blocks in the fattening heifer environment indicated a reduction in their cortisol concentration. Therefore, using salt blocks is recommended as a simple, commercially available, and relatively inexpensive environmental enrichment.

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## KONCENTRACIJA KORTIZOLA U SLINI JUNICA U TOVU DRŽANIH U OBOGAĆENOME OKOLIŠU PRI RAZLIČITIM GUSTOĆAMA NASELJENOSTI

### SAŽETAK

**Cilj istraživanja bio je procijeniti učinak obogaćenja okoliša i različitih gustoća naseljenosti na razinu stresa kod junadi u tovu mjerenjem koncentracije kortizola u slini. Kolorimetrijskim kompetitivnim enzimskim imunotestom analizirano je ukupno 320 uzoraka sline junica tijekom završnoga četveromjesečnog tova. Junice su bile držane pri gustoćama naseljenosti od 3,3 i 4,5 m<sup>2</sup>/životinji u standardnim proizvodnim uvjetima, koji su u pokusnim skupinama dodatno obogaćeni mehaničkom četkom za njegu i blokovima soli. Rezultati istraživanja pokazali su da je koncentracija kortizola na kraju tova bila značajno viša u usporedbi s početnom razinom u svim skupinama junica, bez značajnih razlika među skupinama. Nije bilo značajne povezanosti između korištenja četke za njegu i koncentracije kortizola, no utvrđena je značajna negativna povezanost između korištenja blokova soli i koncentracije kortizola, što upućuje na povoljan učinak ovoga jednostavnog, komercijalno dostupnog i relativno jeftinog obogaćenja okoliša.**

**Ključne riječi:** govedo, kortizol, stres, obogaćeni okoliš, gustoća naseljenosti, dobrobit životinja

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