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Reduction of the olfactory cognitive ability in horses during preslaughter: Stress-related hormones evaluation

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ABSTRACT

As horses may perceive several odour signals of danger at slaughter, application of mentholated ointment to their nostrils may limit their perception of danger. To assess the effect of the application of a mentholated ointment to horse nostrils on the stress response during pre-slaughter handling, plasma levels were evaluated for cortisol, beta-endorphin, epinephrine and norepinephrine prior to and after stunning. Twenty draught-type horses were divided into control (n = 10) and treated (n = 10) groups and a mentholated ointment applied to the nostrils of the treated horses following blood sampling in lairage 45 min prior to slaughter. Treatment did not affect plasma concentrations of beta-endorphin or cortisol but significantly reduced the concentrations of epinephrine and norepinephrine observed in post-stun plasma. These results indicated that mentholated ointment applied to the nostrils of horses pre-slaughter reduced their adrenergic response to the slaughter environment, implying that the stress response may be reduced with this technology.

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1. Introduction

Acute stress immediately pre-slaughter and physiological response to stress (O'Neill, Webb, Frylinck, & Strydom, 2006) are factors that may compromise animal welfare and meat quality. Several studies on cattle (Bourguet et al., 2010; Muchenje, Dzama, Chimonyo, Strydom, & Raats, 2009), pork (Hambrecht et al., 2005; Van de Perre, Permentier, De Bie, Verbeke, & Geers, 2010; Young, Bertram, & Oksbjerg, 2009), sheep and goats (Deiss et al., 2009; Kannan, Kouakou, Terrill, & Gelaye, 2003; Miranda-de la Lama et al., 2009), poultry (Debut et al., 2003; Hindle, Lambooij, Reimert, Workel, & Gerritzen, 2010; Shields & Raj, 2010) and fish (Matos, Gonçalves, Nunes, Dinis, & Dias, 2010; Poli, Parisi, Scappini, & Zampacavallo, 2005) have shown that the impact of pre-slaughter handling procedures can be increased by factors such as restraint, handling, novelty of the pre-slaughter environment, food and water deprivation, adverse weather conditions, changes in social structure, hunger, thirst and fatigue (Apple, Kegley, Galloway, Wistuba, & Rakes, 2005; Grandin, 1997; Mormède et al., 2002).

The high susceptibility of a variety of semi domesticated or free-ranging animal species to stress means that there is a need is to evaluate and consider strategies for preventing pre-slaughter stress (Grigor, Goddard, Littlewood, Warriss, & Brown, 1999; Malmfors & Wiklund, 1996; Pollard et al., 2002; Sabuncuoglu et al., 2011).

Although the horse meat trade is small compared to the beef and swine industries, attention to the welfare of horses destined for slaughter is increasing (Reece, Friend, Stull, Grandin, & Cordes, 2000; Stull, 2001; Werner & Gallo, 2008). In a previous study it was shown that the response of the horse from pre-slaughter to bleeding (getting to the stunning box and stunning phases included) involves strong multifactorial stress (Micera, Albrizio, Surdo, Moramarco, & Zarrilli, 2010). The stress condition in the horse associated with the butchering procedure may be quantified by evaluation of the changes in blood levels of stress related compounds such as cortisol, beta-endorphin, epinephrine, norepinephrine (Micera et al., 2010). The possibility of quantifying the psycho-physical stress level during slaughter may open up ways to improve horse welfare and meat quality (Manteca, 1998).

Horses may perceive at slaughter odour signals of danger (Micera et al., 2010). Mentholated ointment applied on the nostrils could limit the olfactory perception of danger by the animal. To assess effects of the ointment (olfactory barrier) on animal welfare during preslaughter handling, the plasma levels of stress-linked hormones of the neuro-endocrine system were evaluated pre and post slaughter.

2. Materials and methods

2.1. Animals

The work was conducted at the end of Autumn 2008 in a commercial slaughter-house located in Noicattaro (Bari, South-Italy) (outdoor temperature: 7–10 °C) on 20 male Russian Heavy Draught meat horses 3 to 5 years old, with an average weight of 430 kg. They

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were bred in Eastern Europe in open pastures and transported by road in a commercial trailer.

A period of 12 h rest in lairage allowed the animals to recover from transport. This time was chosen to follow the lairage time used in Italy for commercial slaughter of animals travelling long distances, even if the law (EU Directive 93/119/EC) does not suggest a precise lairage time (Papalia, 2003). All horses were subjected to ante-mortem inspection and were healthy. Horses were divided into two groups: C (control) and T (treatment) and mentholated ointment was applied on the nostrils of the treatment group, in the lairage area, 45 min before stunning, immediately after the first blood sampling.

Handling was the same for all the animals fasted for 12 h with access to water. Data shown in this paper only refer to samples recovered from animals that did not suffer coercive treatment.

2.2. Collection of blood samples

From each animal two blood samples were collected: the first was recovered from the jugular vein at 7.00 am, while horses were in lairage, a procedure that took just a few seconds for each horse. The second sample was collected at exsanguination from the hung animal after stunning by captive bolt (MATADOR SS3000, 25 calibre). The distance between the lairage area and the stunning box, approximately 40 m, was covered in nearly 1 min. Animals waited about 45 min before stunning. All procedures were conducted according to the European law on animal protection. Samples, collected using prechilled evacuated tubes (10 mL Vacutainer® BD, UK) containing K_2E, were immediately refrigerated, transported to the laboratory and centrifuged at 800 *g* for 15 min at 4 °C. The recovered plasma fraction was immediately divided into 3 aliquots of 500 μL and stored at −20 °C until analysis.

2.3. Hormone assays

Plasma concentrations of stress related hormones were performed as described by Micera et al. (2010), using commercially available coated-tube enzyme-immunoassay (EIA) kits. The absorbance of each sample was determined at 450 nm (ETI-System Fast Reader S800, Sorin Biomedical, Italy) and the washing was performed using the ETI-System Washer (Sorin Biomedical, Italy).

Plasma concentrations of epinephrine and norepinephrine were measured in duplicate utilising 2-Cat EIA kit 07L-114602 MP Biomedicals Inc. (NY, USA). The hormone assay had intra-assay coefficients of variation (CV) of: epinephrine 14.30%, norepinephrine 11.75%; while the inter-assay CVs were: epinephrine 10.95%, norepinephrine 12.95%. The sensitivity was 0.011 ng/mL for epinephrine and 0.044 ng/mL for norepinephrine.

Plasma cortisol concentrations were analysed in duplicate using EIA kit code 5580QN M.B.S. Medical Biological Service (Milano, Italy). The hormone assay had intra- and inter-assay CVs of 5.7% and 3.2%, respectively. The sensitivity of the cortisol kit was 1 ng/mL.

Plasma beta-endorphin concentrations were measured in duplicate utilising EIA kit S-1245 (EIAH8609) for camel, bovine, ovine beta-endorphin, with 100% cross-reactivity with equine beta-endorphin, Peninsula Laboratories Inc. (San Carlos, California, USA). The hormone assay had intra- and inter-assay CVs of <5% and <14%, respectively. The sensitivity of the beta-endorphin kit was 0.03 ng/mL.

Specificity, for each hormone, was demonstrated by parallelism of curves prepared with standards and serially diluted equine plasma pools (data not shown).

To minimise variability, all samples were processed in 1 day using the enzyme-immunoassay procedure recommended by the supplier.

2.4. Statistical analysis

Data are means ± standard deviations of the mean (SD). Statistical analysis was performed by two way ANOVA to determine hormonal variations before and after stunning. Results were taken to be statistically significant at the $P<0.05$ level.

3. Results

Plasma concentrations of cortisol, beta-endorphin, epinephrine and norepinephrine of C and T, measured during lairage (t1) in the pre-slaughtering area and during exsanguination, after stunning (t2), are shown in Fig. 1, panels A, B, C and D, respectively.

After stunning, in both C and T, the level of the hormones, except for beta-endorphin, increased compared to the level measured during lairage. The increase was statistically significant: $P<0.05$ for cortisol (panel A) and $P<0.001$ for epinephrine (panel C) and norepinephrine (panel D). The increase of plasma beta-endorphin concentration in C was not statistically significant, whereas there was no increase of concentration in T (panel B).

Difference in plasma cortisol concentration (panel A) between C and T, after stunning (t2), wasn't statistically significant. Plasma cortisol value in C measured pre-slaughter was 242.58 ng/ml (±156.08), whereas in T it was 259.22 ng/ml (±148.16), while the value at bleeding in C was 377.36 ng/ml (±191.85), and in T was 455.07 ng/ml (±43.04).

Plasma beta-endorphin concentration showed, in C, an increasing trend from 0.98 ng/ml (±0.39) during lairage (t1) to 1.20 ng/ml (±0.20) after stunning (t2), whereas in T the concentration was 0.95 ng/ml (±0.13) in t1 and 0.93 ng/ml (±0.15) in t2. There were no significant differences between C and T in t2.

Plasma levels of both epinephrine and norepinephrine increased markedly and similarly in response to stressors. After stunning (t2) increases in epinephrine (panel C) and norepinephrine (panel D) concentrations between the two groups C and T were different ($P<0.05$).

T plasma epinephrine value in C at lairage (t1) was 0.35 ng/ml (±0.17) and in T was 0.25 ng/ml (±0.11). After stunning (t2) the concentration of the hormone in C increased to 8.98 ng/ml (±1.52) and in T to 5.21 ng/ml (±1.05), so there was a considerably reduced increase in this group.

The plasma norepinephrine content in C pre-slaughter (t1) was 0.67 ng/ml (±0.43), and in T was 0.85 ng/ml (±0.34); during bleeding (t2) the values in C and T were 6.84 ng/ml (±1.40) and 4.16 ng/ml (±1.03) respectively; the increase in norepinephrine concentration was significantly lower in T.

4. Discussion

Equine adaptive responses to physical and/or psychological stressful stimuli depend on several system interactions: activation of the sympathetic nervous system (SNS) (Sanders & Straub, 2002), of the hypothalamic–pituitary–adrenocortical (HPA) axis (Mormede et al., 2007; Nagata et al., 1999) and of the endogenous opioid system (Luna & Taylor, 2001; Vaanholt, Turek, & Meerlo, 2003). The present results show that stress-linked hormonal plasma concentrations in C and T, except for beta-endorphin, increased significantly after stunning ($P<0.05$ for cortisol, $P<0.001$ for epinephrine and norepinephrine). These results were in accordance with a previous study, which evaluated strong multi-factorial stress in horses during the time from pre-slaughter to bleeding (Micera et al., 2010).

It is interesting to note that the use of the mentholated ointment applied on the nostrils, to limit the olfactory perception of danger by the animal, did not effectively reduce the increase of cortisol after stunning (t2) in T, there were no significant changes between C and T. This could be explained by the complex mechanisms involved in olfactory modulation, which involves structures used for the production of glucocorticoids. Indeed the locus coeruleus has a behaviorally meaningful role within the olfactory bulb of adult animals engaged in olfactory learning tasks (Day, Campeau, Watson,

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