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Stress-related hormones in horses before and after stunning by captive bolt gun

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ABSTRACT

In this work the slaughter-linked plasma modifications of some stress-related hormones in horses subject to standardized butchering procedures were investigated in order to highlight the compromised animal welfare during pre-slaughter handling. During pre-slaughter, animals show strong hardship behavioural patterns, probably due to being under life-threatening conditions. Blood samples from 12 male horses, ageing from 3 to 5 years, were collected before slaughtering in lairage, and during exsanguination after stunning. Catecholamines, cortisol and beta-endorphin concentrations were assessed in plasma samples by EIA. Results show that plasma beta-endorphin concentration did not increase significantly after stunning, while cortisol ($P < 0.05$) and catecholamines ($P < 0.001$) increased significantly. The ratio between the plasma level of norepinephrine and epinephrine decreased significantly ($P < 0.001$) during the time considered for observation underlining a greater involvement of adrenal medulla in the stress response. Moreover these results suggest that, under stress, the release of beta-endorphin could be different from that of ACTH.

1. Introduction

Countless works describe the modifications induced in behaviour in horses by different commercial transports both as runs and destinations, (Smith, Jones, Carlson, & Pascoe, 1994; Waran, Robertson, Cuddeford, Kokoszko, & Marlin, 1996) and on haemato-chemical and haematological parameters (Anderson, De Bowes, Nyrop, & Dayton, 1985; Crisman, Hodgson, Bayly, & Liggitt, 1992) including hormone levels (Baucus, Ralston, Nockels, McKinnon, & Squires, 1990; Ferlazzo, Fazio, Murania, & Piccione, 1993). All data underlines that the response to stressors is related to type and length of the transport (Fazio & Ferlazzo, 2003), training (Rivera, Benjamin, Nielsen, Shelle, & Zanella, 2002) and interaction with humans, and also each horses own previous experience, age, sex, temperament, breed, health and environmental conditions (Mormede et al., 2007; Stull, 1999; Stull & Rodiek, 2000). On the contrary, changes induced in horses in events of restraint and strong environmental restriction, such as that they experience during the short run from the lairage area to the restraining stunning box and during stunning, are much less investigated.

Considering that plasma cortisol, epinephrine, norepinephrine and beta-endorphin are believed to be the main biological indicators of stress conditions in horses (James, Horner, Moss, & Rippon, 1970; Snow, Harris, Macdonald, Forster, & Marlin, 1992), the purpose of this study was to evaluate stress responses in animals on environmental restraints, that are aware of being under life-threatening conditions. Plasma levels of the stress-linked hormones of the neuro-endocrine systems were monitored during the pre-slaughter phases from lairage to exsanguination. Further stressor treatments of animals such as violent coercions (i.e., the use of an electric goad, or beating up with a stick) were avoided the slaughtering operatives having received an adequate training.

2. Materials and methods

The work was conducted at the end of Autumn 2004 in a commercial slaughter-house located in Noicattaro (Bari, South-Italy) (outdoor temperature: 6–8 °C). The research was carried out on 12 male Russian Heavy Draught meat horses assigned to human consumption aged from 3 to 5 years, with an average weight of 450 kg. They were bred in East Europe in open pastures and transported by road in a commercial trailer for a period of about 36 h to the slaughter-house, where they arrived at least 12 h before the trial started.

This time was chosen to follow the lairage time used in Italy for commercial slaughter of animals coming from long distance, even if the law (EU Directive 93/119/EC) does not suggest any precise lairage time (Papalia, 2003). A period of rest in lairage was observed to allow animals to recover from transport. All horses were subjected to ante-mortem inspection and all were healthy. Handling was the same for all animals fastened for

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12 h with access to water. Data shown in this paper only refer to samples recovered from animals that did not suffer coercive treatment.

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 bullet (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 for about 45 min before stunning. All procedures were conducted according to the European law on animal protection cited above during slaughter and stunning.

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 aliquots and stored at −20 °C until analysis.

Plasma hormone levels were assessed using commercially available coated-tube enzyme-immunoassay (EIA) kits. These competitive ELISA kits use the microtiter plate format. All microtiters plates were washed using the ETI-System Washer (Sorin Biomedical, Italy) and the absorbance of each samples was determined at 450 nm (ETI-System Fast Reader S800, Sorin Biomedical, Italy). Quantification of unknown samples is achieved by comparing their absorbance with a reference curve prepared with known standard concentrations.

Plasma concentrations of epinephrine and norepinephrine were measured in duplicate utilizing 2-Cat EIA kit 07L-114602 MP Biomedicals Inc. (NY, USA). These catecholamines were extracted using a cis-diol-specific affinity gel, then acylated to *N*-acyladrenaline, *N*-acylnoradrenaline and after this converted enzymatically during the detection procedure into *N*-acylmetanephrine and *N*-acylnormetanephrine, respectively. The hormone assay utilised had intra-assay coefficients of variation (CV) of: epinephrine 14.30%, norepinephrine 11.75%; while the inter-assay CV 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 utilised had intra- and inter-assay CV of 5.7% and 3.2%, respectively. The sensitivity of cortisol kit was 1 ng/ml.

Plasma beta-endorphin concentrations were measured in duplicate utilizing 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). Equine beta-endorphin has an amino acid sequence identical to that of ovine, bovine and camel beta-endorphin except for substitution of the threonine residue at position 6 by serine (Li et al., 1981). The hormone assay utilised had intra- and inter-assay CV of <5% and <14%, respectively. The sensitivity of beta-endorphin kit was 0.03 ng/ml.

Specificity, for each hormone, was demonstrated in our laboratory by parallelism of curves prepared with standards and serially diluted equine plasma pools.

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

2.1. Statistical analysis

Data are shown as mean ± standard deviation of the mean (SD). Statistical analysis was performed by one way ANOVA to determine hormonal variations before and after stunning. The level of significance was set at $P < 0.05$.

3. Results

Plasma concentrations of epinephrine, norepinephrine, cortisol and beta-endorphin, measured during the lairage time (t_1) in the pre-slaughtering area and during exsanguination, after stunning (t_2), are shown in the Fig. 1, panel A, B, C and D, respectively. After stunning, the level of each examined hormone increased compared to the level measured during the lairage stay. The increase was statistically significant $P < 0.001$ for epinephrine (panel A) and norepinephrine (panel B) and $P < 0.05$ for cortisol (panel C). The increase of plasma beta-endorphin concentration was not statistically significant (panel D).

Plasma epinephrine content was 0.3 ng/ml (±0.19) at lairage (t_1). After stunning (t_2) the concentration of the hormone was 9.25 ng/ml (±1.67), an increase of 30 times. The levels of norepinephrine detected in the two samples showed an increase similar to that of epinephrine: the basal value at t_1 was 0.6 ng/ml (±0.52 ng/ml) and it increased up to 7.6 ng/ml (±1.44) during bleeding (t_2), rising the value by over 12 times. The value of cortisol measured during pre-slaughter was 234.01 ng/ml (±167.29), whereas the value at bleeding was 358.78 ng/ml (±190.38), so its value increased more than 50%.

The mean beta-endorphin plasma concentration showed an increasing trend from 1.03 (±0.32) ng/ml at t_1 to 1.17 (±0.32) at t_2.

4. Discussion

In the last years, a strong ethical sensitivity to animal welfare, in all phases of their life and environment, has occurred. Utilitarian motivations have extended this sensitivity to farm livestock. The negative impact of ante-mortem stress on meat eating quality is well known (Apple et al., 2005; Hambrecht et al., 2004; Kannan, Kouakou, Terrill, & Gelaye, 2003; Ljungberg, Gebresenbet, & Aradom, 2007; Schaefer, Dubeski, Aalhus, & Tong, 2001) and strengthens the hypothesis that a lower animal stress during breeding, transport and slaughtering phases improves meat and by-product quality with positive economic and qualitative influences (Casoli, Duranti, Cambiotti, & Avellini, 2005).

It is easy to understand that each animal perceives, at slaughter, several signals of danger, such as odours, sights and hearings; in fact for these animal's images, sounds and mostly smell constitute a very rich perceptive universe which is used to regulate social and sexual behaviours and to ensure the survival in dangerous situations (Micera & Zarrilli, 2004). Hardship behavioural patterns are evident during moving and staying in the stunning box: reluctance to proceed, restlessness, unsure walking with continuous ears movement and frequent snorting.

In horses adaptive responses to physical and/or psychological stressful stimuli also depend on the interaction of several systems. The SNS component of the stress response results in secretion of norepinephrine and epinephrine into the bloodstream. The HPA axis regulates production and release of glucocorticoids. Beta-endorphin seems to be involved in pain perception, acquisition of avoidance behaviours and in physiological, behavioural and emotional responses to averse stimuli (Clark, Rager, & Calpin, 1997; Vaanholt, Turek, & Meerlo, 2003; Zelena, Kiem, Barna, & Makara, 1999).

Plasma levels of glucocorticoids, catecholamines and beta-endorphins are among the most frequently used parameters to study short-term welfare problems such as those encountered during exercise, transport and lairage (Manteca, 1998).

In our work we found a significant increase ($P < 0.05$) of cortisol after stunning with captive bullet that strengthens the idea that this hormone is more important as stress/excitement/hardship response than as a weariness signal function. On the other hand it