Livestock Science 173 (2015) 106–110

Contents lists available at ScienceDirect

Livestock Science

journal homepage: www.elsevier.com/locate/livsci

Short communication

Evaluation of secondary stress biomarkers during road transport in rabbit

F. Fazio, S. Casella*, E. Giudice, C. Giannetto, G. Piccione

Department of Veterinary Sciences, Faculty of Veterinary Medicine, Polo Universitario Annunziata, University of Messina, 98168 Messina, Italy

ARTICLE INFO

Article history:
Received 16 September 2014
Received in revised form
7 January 2015
Accepted 8 January 2015

Keywords:
Rabbit
Rectal temperature
Road transport
Secondary stress biomarkers
Total proteins

ABSTRACT

The aim of this study was to evaluate the effect of road transport, excluding the effect of cages, on rectal temperature (RT), glucose, lactate, packed cell volume (PCV) and total proteins (TP) in rabbits. The animals were divided into three groups of 10 subjects: Group A was transported into plastic transport crates by road for 2 h over a distance about 160 km with an average speed of 80 km/h, Group B was untransported and placed into plastic transport crates and Group C was untransported and placed into battery-style cages. RT, glucose, lactate, PCV and TP were measured before (*T0*) and after the road transport (*T2*) as well as after 6 (*T6*) and 24 h (*T24*) rest time in Groups A–C. The GLM (General linear model) Repeated Measures procedure, followed by Duncan multiple post-hoc comparison test, showed statistically significant differences among the Groups A–C ($P < 0.0001$) and a significant effect of sampling time ($P < 0.0001$) on RT, glucose, lactate and PCV in Group A. The results suggest that in rabbits, the changes of rectal temperature, together with the secondary stress markers, play an important role in providing complementary information for the assessment of transportation stress suggesting that these modifications are useful not only for monitoring stressful conditions but also for evaluating health and animal welfare.

1. Introduction

Transport may be considered as an extension of handling on the farm; however this housing is now in movement through changing environments that challenge the adaptive mechanisms of the animal. Actually, even though strict welfare rules for transport of animals are enforced and numerous recommendations on transport are worldwide known, the road transport is a critical point that has been little studied, especially for rabbits. It is known that transport is one of the main causes of stress, raising considerable interest (Friend, 2011). During the transport, the animals are generally exposed to physical, psychological and physiological stressors that disrupt homoeostasis and metabolism of animals (Mohammadi et al., 2007; Friend, 2011). In order to restore homoeostasis and to promote survival, animals respond to stressful stimuli through a rapid cascade of endocrine secretions (Sapolsky et al., 2000) and a pattern of behavioural, neural, immune, haematological and metabolic changes (Knowles and Warriss, 2000; Todd et al., 2000; Warriss, 2000; Muir, 2004; Giannetto et al., 2011; Casella et al., 2012; Piccione et al., 2012). This often results in increased susceptibility to diseases (Stull, 1999; Earley and O'Riordan, 2006). Therefore, the degree of stress response often correlates with the overall health of an individual (Romero, 2004; Wasser et al., 2000); thus, researchers use the behavioural and physiological variability to measure and monitor animal

* Corresponding author. Tel.: +39 090 3503520; fax: +39 090 3503975.
E-mail address: stefania.casella@unime.it (S. Casella).

http://dx.doi.org/10.1016/j.livsci.2015.01.006
1871-1413/

welfare. Particularly in rabbits, the physiological response to stress has been evaluated analysing the association between the glucocorticoid measurements and various components of individual fitness, including body condition and survival in the wild (Cabezas et al., 2007). The response to thermal stress in the context of transport has been also evaluated to monitor the welfare of rabbits during their transport to the slaughterhouse (de la Fuente et al., 2007). In addition, changes in electrolytes, metabolites and enzymes of rabbits subjected to transport under potential transport-related stressors have been evaluated (Nakyinsige et al., 2013). Some researchers showed that stress conditions play a central role in the release of hormones and in the stimulation of the hypothalamic–adrenal-axis (Möstl and Palme, 2002), but also in the activation of a secondary response as consequence of the released hormones, that causes changes in the blood and tissue chemistry (Everds et al., 2013). Particularly, some authors studied in rabbits the variations in plasma levels of glucose and lactate, packed cell volume (PCV) and total proteins (TP) whose values elevated by stressors may be associated with animal welfare (Mazzone et al., 2010; Vignola et al., 2008; Choe and Kim, 2014). Previous studies have shown the relationship between stress hormones and transport in rabbit (de la Fuente et al., 2004, 2007; Liste et al., 2008; Mazzone et al., 2010), but there is little information on the relationship between the secondary biomarkers of stress and transport of rabbits (de la Fuente et al., 2004, 2007; Liste et al., 2008; Nakyinsige et al., 2013).

The aim of this work was to study the modifications of rectal temperature, total proteins and some secondary stress biomarkers (glucose, lactate and PCV) in rabbit during the road transport practice in order to identify the impact of transport stress on parameters that could be useful to improve the health and animal welfare excluding the effect of cages.

2. Materials and methods

The study was carried out on New Zealand White rabbits from the same farming industry. Thirty male rabbits, clinically healthy, with a mean body weight of 2.5 ± 0.3 kg and 12-week old, were used. The rabbits were given access to pelleted feed (proximate analysis: 14.5% crude protein, 7.2% crude fibre, 7% fat, 0.8% calcium and 0.4% phosphorus) and water ad libitum.

Ten rabbits, placed into plastic transport crates, were transported covering a distance of about 160 km within 2 h with an average speed of 80 km/h (Group A) and 20 rabbits were not subjected to transportation. Particularly, the untransported rabbits were divided into Group B (10 rabbits) and Group C (10 rabbits). Each animal of the Group B was placed into plastic transport crates ($20 \times 35 \times 30$ cm^3, length × width × height) (one animal per cage), and the animals of the Group C were placed into battery-style cages (one animal per cage). The transport, commenced at 09.00 h for a 2 h period, involved a combination of road surfaces ranging from small country lanes (5 km) and secondary roads (25 km) to motorways (130 km). The transport truck, as generally used in Italy, was uncovered and it had an oilcloth roof and the side walls were open bars. The animals of Group A were placed in 10 plastic transport crates on the same side of the truck. After the transport, the animals of Group A were housed into plastic transport crates for a 24 h post-transport recovery period.

The animals were transported at the ambient temperature of 20 °C and relative humidity of 69% at loading time and during the first stage of the transport, and at 21 °C and 71% in the final stage of the transport and unloading. Temperature and relative humidity inside the vehicle during the transportation ranged from 23 to 25 °C and 80% to 83%, respectively. For all experimental periods, temperature and relative humidity were continuously recorded with a data logger (Gemini, Chichester, West Sussex, UK). The animals of Groups B and C were located at the destination site of transported rabbits in the same environmental conditions. All animals of Group A were transported in accordance with Directive 1/2005/EC.

On all rabbits rectal temperature was recorded before the transport (*T*0), immediately after the transport (*T*2), 6 (*T*6) and 24 h (*T*24) after the transport, with a calibrated electronic thermometer with resolution of 0.1 °C (Model HI-92740, Hanna Instruments, Bedfordshire,UK) inserted into the rectum at the depth of 1 cm. For the evaluation of blood parameters during experimental period (*T*0, *T*2, *T*6 and *T*24), the blood was taken from the central ear vein of each rabbit. Blood glucose and lactate levels were immediately assessed using a portable blood glucose (ACCU-Chek Active, Roche Diagnostics GmbH, Germany) and a blood lactate analyzer (Accusport, Boehringer, Germany), respectively. For PCV and TP measurements, the blood was taken with a 2.5 ml syringe and 22-ga needle and it was placed into two different blood collecting tubes (Aptaca, Asti, Italy): test tubes with K_3-EDTA (1 ml of blood) and test tubes with Lithium Heparin (1 ml of blood). On samples with K_3-EDTA, PCV was assessed using an automatic counter (HeCoVet C, SEAC) and on samples with Lithium Heparin, total protein concentrations were determined with commercially available kits by means of a UV spectrophotometer (model Slim SEAC, Firenze, Italy). All samples were assayed in duplicate by the same person each time.

Protocols of animal husbandry and experimentation were reviewed and approved in accordance with the standards recommended by the *Guide for the Care and Use of Laboratory Animals* and by Directive 86/609 CEE.

All results were expressed as mean ± standard deviation (SD). Data were normally distributed ($P < 0.05$, Kolmogorov–Smirnov's test). The GLM (General linear model) procedure of the SPSS version 13.0 statistical package (SPSS, 2006) was used to determine the differences between the experimental and control groups, and the statistically significant effect of sampling time (*T*0, *T*2, *T*6 and *T*24). Values of $P < 0.05$ were considered statistically significant. Duncan multiple post-hoc comparison test was applied.

3. Results

Statistical analysis showed a significant difference between the untransported animals and rabbits subjected to road transport ($P < 0.0001$), and the influence of sampling time on rectal temperature ($P < 0.0001$), glucose ($P < 0.0001$), lactate ($P < 0.0001$), PCV ($P < 0.0001$) and TP ($P < 0.0001$) in Group A. All studied parameters showed