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HAEMATOLOGICAL PARAMETERS AS INDICATORS OF TRANSPORT STRESS IN RABBITS

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ABSTRACT

The purpose of our investigation was to evaluate the effect of transport to the slaughterhouse on some haematological stress indicators in rabbits. All the animals used came from the same farm and were randomly chosen from those that reached the end of their productive cycle (82 days old). A total of four journey session were performed. Each journey lasted 100 minutes. Moreover, for each journey, the same lorry driven by the same transport operator, was used. In order to assess stress condition, blood samples from 80 male rabbits (20 animals per journey session) were collected two days before each transport session and at the de-bleeding in the slaughterhouse. Haematological and biochemical variables were analyzed: packed cell volume (PCV) did not differ significantly after transport while a significant neutrophilia ($P<0.001$), lymphocytopaenia ($P<0.001$) with a significant increase in the N/L ratio were observed in all transported rabbits. A significant increase ($P<0.001$) in serum glucose concentration following transport, was found. Furthermore, total protein, albumin and osmolality increased significantly in all rabbits. These findings underlined a moderate dehydration caused by transport that was evidenced also by the increasing in Na and K serum concentrations. A significant upsurge in serum aspartate aminotransferase (AST) ($P<0.01$), alanine aminotransferase (ALT) ($P<0.001$) and creatine kinase (CK) activities ($P<0.001$) were recorded in all rabbits after transport.
A twofold increase in serum corticosterone concentration (6.23 *vs.* 14.88 ng/mL; $P<0.01$) evidenced the stress condition experienced by all rabbits during transport.The altered levels of some of the analyzed blood variables, lead to the conclusion that, as in other species, haematological parameters may be usefully employed to highlight the stress condition of rabbits during transport.

Key words: Rabbit, Transport, Stress indicators, Welfare

INTRODUCTION

Transportation is an important activity of the farming industry. It is also a topical controversial area of animal welfare and several authors have confirmed that transportation for short or long periods can impose stress on animals (Marìa *et al.*, 2006; Liste *et al.*, 2008); particularly, pre-transport and transport conditions may cause injury, reduce performance, cause increased morbidity and mortality rates and consequently substantial economic losses due to loss of liveweight and poor meat quality (Lambertini *et al.*, 2006; Minka and Ayo, 2007). In order to reduce the adverse effects on food animals and the economic losses encountered during animal transportation, the EU legislators set out the Regulation 1/2005/EC.

Transport involves several potential stressors such as rough handling during loading and unloading, deprivation of food and water, poor vehicle design, poor road conditions, extremes of temperature and humidity, overcrowding, mixing different species and age groups, high air velocity, noise, motion, vibration and length of the journey (Buil *et al.*, 2004; Verga *et al.*, 2009). The stress reactions can overload the body systems and cause reduction in fitness of the animal by inducing dysfunctions of the pituitary, adrenal and thyroid glands. It has been demonstrated that transportation induces in livestock changes in the blood composition as well as other body parameters like heart rate, electrolytes, hormones, metabolites, enzymes, liveweight and meat quality (Mitchell *et al.*, 1988).

In rabbits few studies have focused on the effect of transport from farm to the slaughterhouse on welfare of commercial rabbits. European Food Safety Authority (EFSA, 2004) have indicated the size and height of the crates, type of floor, mixing unfamiliar animals, thermal stress and lack of ventilation as the most important hazard factors involved in transport stress on rabbits. New scientific evidence have confirmed these conclusions, and also investigated their impact in relation to other factors, such as loading methods and the position of the crates within the truck (Mazzone, *et al.*, 2010; Liste *et al.*, 2008). The purpose of our study was to assess whether some hematological parameters in rabbits could be used as indicators of stress during transport to the slaughterhouse, as for other species, to obtain new evidence that could contribute to improve the current legislation on animal welfare during transport.

MATERIALS AND METHODS

Animals and experimental design

Animal handling and transport followed the recommendations of the Regulation 1/2005/EC. To evaluate if some hematological parameters in rabbits could be used as indicators of stress during transport a total of 80 animals were used. All the animals came from the same farm and were randomly chosen from those that reached the end of their productive cycle (82 days old). A total of four journey session were performed. The transport truck, as generally used in Italy, was uncovered and had an oilcloth roof and the side walls were open bars. A total of 128 plastic transport crates (98 x 52 x 24 cm, length x width x height) provided with loading doors were already on the truck. The number of rabbits transported for each journey session was about 1500, filling the capacity of the truck. Each journey lasted 100 minutes. Moreover, for each journey, the same lorry driven by the same transport operator was used.

Blood analysis

In order to assess changes in blood parameters related to stress condition, two days before each transport session individual blood samples (5 ml) from 80 male rabbits (20 animals per journey session) were collected from the central ear vein with a 5-ml syringe and 22-ga needle and placed into EDTA tube and in a serum separator tube (Terumo Venoject, Belgium). Individual blood samples were then collected from the same rabbits at the de-bleeding in the slaughterhouse. Samples were kept refrigerated until arrival at the laboratory for immediate processing. Haematological parameters: red blood cells (RBC), packed cell volume (PCV), haemoglobin (Hb) and white blood cells (WBC) were analysed with an automatic counter (ADVIA®120, Deerfield, IL - USA). Serum samples were quickly obtained by centrifugation at 3500 x g for 10 min and divided in two aliquots and immediately frozen (- 80 °C). One aliquot was used for analysis of glucose, total proteins (TP), sodium (Na), potassium (K) and enzymatic activities of creatine kinase (CK), lactate dehydrogenase (LDH), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) with an Olympus AU 400 auto-analyzer. On the second aliquot serum corticosterone levels were determined with a commercial competitive ELISA kit (Neogen Corp., USA). To perform the assay, 0.1 ml of serum was used and the determination was carried out as recommended by the kit supplier. Final absorbance was measured in a microplate reader (DV 990 BV6, GDV S.r.l., Rome, Italy).

Statistical analysis

Data were tested for normal distribution using the K S – Liliefors test. Blood parameters obtained before and after transport were compared using a Repeated Measures GLM of the SPSS 13.0 statistical package (SPSS, 2006), including the transport session as random factor. The effect of the transport session is not reported because not significant. Results are presented as means, and variance is expressed as standard error.

RESULTS AND DISCUSSION

The mean values of haematological and biochemical variables investigated in relation to transport are presented in Tables 1 and 2.

Table 1 Effects of transport on haematological parameters of rabbits (mean values)

		Sampling		P values	Standard Error
		Before Transport	After Transport		
Number of samples		80	80		
Red blood cells	(10^6/µL)	6.54	6.27	0.651	0.035
Haemoglobin	(g/dL)	12.70	12.56	0.885	0.304
Packed cell volume	(%)	40.48	39.57	0.141	0.313
White blood cells	(10^3/µL)	11.11	13.41	<0.001	0.476
Neutrophils	(%)	35.40	50.96	<0.001	1.041
Lymphocytes	(%)	56.81	38.56	<0.001	1.059
Neutrophil:lymphocyte ratio		0.67	1.50	<0.001	0.069

Haematological parameters commonly used as indicators of stress during transport are presented as a contrast between pre and post-transport results and it is possible to observe that some of the variables considered were significantly altered by transport. In the present work PCV after transport did not differ significantly from values detected at the farm, probably because of the short duration of the journey. This finding agrees with Liste *et al.* (2008), who did not find any increase in PCV in commercial rabbits transported at different levels in multi floor cage rolling stands. The effect of transport on PCV values would be dependent on the stress produced by loading and handling the animals prior to transport and to the duration of transport. Increases in PCV values were observed with different journey times in other species (Tadich *et al.*, 2005; Parker *et al.*, 2007), which may suggest dehydration, but Knowles *et al.* (1999) noted a PCV decrease in transported calves that could indicate a progressive habituation of animal to being handled.

Table 2 Effects of transport on biochemical parameters of rabbits (mean values).

		Sampling		P values	Standard Error
		Before Transport	After Transport		
Number of samples		80	80		
Glucose	(mg/dL)	139.98	160.64	<0.000	3.497
Blood Urea Nitrogen	(mg/dL)	32.40	32.66	0.563	0.539
Aspartate aminotransferase (AST)	UI/L	25.39	32.58	0.001	2.096
Alanine transferase (ALT)	UI/L	31.84	38.04	<0.000	0.641
Creatine phosphokinase (CK)	UI/L	885.86	2905.76	<0.000	195.837
Lactate dehydrogenase (LDH)	UI/L	229.69	234.96	0.881	35.312
Total protein	(g/dL)	5.799	6.014	0.003	0.072
Albumine	(g/dL)	3.705	3.889	0.001	0.054
Potassium	(g/dL)	5.543	7.54	<0.000	0.151
Sodium	(mmol/L)	139.43	149.56	<0.000	1.176
OsmT	(mOsm/L)	309.28	334.79	<0.000	2.426
Corticosterone	(ng/mL)	6.23	14.88	0.001	2.465

White blood cell (WBC) count and differential were significantly altered by transport, as evidenced by the significant neutrophilia (P<0.001) and lymphocytopaenia (P<0.001) observed in all rabbits at slaughter as a result of the endogenous release of corticosteroids. Another measure useful to evidence the sustained effect of stress is the neutrophils/lymphocytes (N:L) ratio, which increased significantly after transport. The N:L ratio has been used as a reliable index of stress in birds (Scope *et al.*, 2002) and other livestock (Kannan *et al*., 2000). In the present study, a significant increase in the N/L ratio was evidenced in all rabbits. These results indicate that, for rabbits, even a short transport significantly influences haematological parameters that can so be used as stress indicators. Moreover, a significant upsurge in serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) and creatine kinase (CK) activities was observed in all rabbits (Table 2).

The increase in AST, ALT and CK activities could be interpreted as an index of cell muscle damage and muscle fatigue. In particular CK is the most sensitive enzyme that shows muscle damage and even intense muscle activity. Increased CK activity reflects the increased muscle fatigue related to transport (EFSA, 2004). In a previous work, (Vignola *et al.,* 2008), we found a significant upsurge of AST and CK activities in rabbits transported to the abattoir, independently of the crate position or loading method on the truck, evidencing so that rough loading method did not act as a major stressor with respect to normal transport and handling for rabbits. In other livestock conversely, Kannan *et al.* (2003) found that vigorous physical activity, such as herding, loading and unloading procedures were more important than transportation itself in determining plasma CK activity. A significant increase in serum glucose concentration following transport, was also found. Kannan *et al.* (2003) evidenced a rapid increase in plasma glucose concentration in goats after a short transport directly correlated to that of plasma cortisol. On the contrary, Liste *et al.* (2006) recorded a significant decrease in plasma glucose levels in rabbits transported to the abattoir suggesting that this was probably due to an increase in glucose consumption, resulting from the stress of transport. Knowles *et al.* (1995) reported that livestock transported for up to 24 h tended to show an increase in plasma glucose during the journey, rather than a decrease that would indicate metabolic exhaustion. This plasma glucose increase was most probably due to the initial stress response evidenced by an elevation of the cortisol concentration, inducing a mobilization of body energy reserves (Kannan *et al.*, 2000).

Total serum proteins and serum osmolality can both be used as measures of dehydration and the stress condition (EFSA, 2004). As previously stated, PCV was not influenced by transport. Usually, dehydration is associated with an increase in PCV and plasma protein concentration. In the present study, only total protein, albumin and osmolality increased significantly. These results suggest a moderate dehydration caused by transport that is also evidenced by the rise in Na and K serum concentrations. As evidenced in several studies, the events occurring prior to slaughter lead to physical stress as well as psychological stress. Animals respond physiologically to the perception of stressful stimuli with the secretion of glucocorticoids (GC, cortisol or corticosterone) from the adrenal glands. The degree of stress response activation often correlates with the overall health of an individual, and the quantification of GC levels is often used to study the health status of animal populations. Corticosterone is the major adrenal glucocorticoid secreted by the European rabbit.

Transport and handling are often reported to evoke an increase in adrenal cortex responses and thus viewed as stressors (EFSA, 2004; Vignola *et al.,* 2008; Mazzone *et al.,* 2010). In the present work serum corticosterone increased significantly due to the activation of the stress response system. In particular, a twofold increase in serum corticosterone was observed in all rabbits following transport. Liste *et al*. (2006) evidenced that position on the truck could affect serum corticosterone level. These authors underlined that rabbits located in the top of the transport truck had lower corticosterone concentration than those located in the middle or in the bottom of multi floor cage rolling stands suggesting that the vertical position may affect the welfare of rabbits, particularly of rabbits placed in the bottom. The same authors related this finding to a possibly higher temperature in the bottom portion; Vignola *et al.* (2008) did not confirm these findings and found that independently of the crate position on the truck, corticosterone level increased in all animals transported to the abattoir. By contrast, the same authors found that plasma corticosterone levels increased significantly during transport following the rough loading method suggesting that it represents a severe stress for the animals.

CONCLUSIONS

The results obtained showed that the haematological parameters analyzed were significantly influenced by transport to the abattoir. As recently underlined by the "Scientific Opinion Concerning the Welfare of Animals during Transport", in order to improve EU legislation for rabbit protection during transport, further research is needed to evaluate the effects of environmental condition, thermal limits and space allowance in the truck. We can conclude that haematological parameters may be usefully employed to highlight the stress condition of rabbits during transport.

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