EFFECTS OF LOADING METHOD AND CRATE POSITION ON THE TRUCK ON SOME STRESS INDICATORS IN RABBITS TRANSPORTED TO THE SLAUGHTERHOUSE

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ABSTRACT

The aim of this study was to determine the effects of loading method and crate position on the truck on some stress indicators in commercial rabbits transported to the slaughterhouse. On two journeys in July a total of 192 animals (82 days old) were transported to the slaughterhouse during the morning (9.00 a.m.) for a mean transport time of 100 min. The transport truck was uncovered, with a total of 128 fixed plastic transport crates, for an overall capacity of 1500 subjects. To evaluate the effects of crate position, at each session, 96 animals were distributed at random in 8 crates on the same side of the truck (12 animals per cage, 57.7 kg/m^2) as follows: 24 animals in 2 top front (TF) crates, 24 animals in 2 bottom front (BF) crates, 24 in 2 top rear (TR) crates and 24 in 2 bottom rear (BR) crates. In the middle of TF, BF, TR and BR crates four data-loggers were placed for temperature (T°) and relative humidity (%RH) control. To evaluate different loading methods one cage from each position was loaded in a smooth way (S: 12 rabbits from the farm crates were placed in a wide trolley and carried gently into the transport cage - loading time for 48 subjects: 12 min.) while the other was loaded in a rough way (R: rabbits from four crates were carried all together in the same trolley and loaded hurriedly - loading time for 48 subjects: 4 min.). All rabbits were individually weighed before transport and at unloading. To assess some stress indicators, blood samples were collected from 40 male rabbits (20 per journey: 5 per truck position, 10 per loading method) on the farm 2 days before loading (basal level) and at slaughter during exsanguinations. The TR crates showed the highest mean temperature and the lowest relative humidity ($P<0.001$) while the other cages on the truck differed only in humidity. No effects on weight losses during transport could be ascribed to loading methods or crate position in the truck. Corticosterone showed a tendency to increase from basal levels in all animals, the increase being significant only during transport using the rough loading method. Neutrophilia and lymphocitopenia were significant for all rabbits, independently of their position in the truck or the loading method. Packed cell volume never differed significantly among groups. Rabbits transported in TR crates (with higher mean temperature) showed a significant augmentation of total protein level, as a possible consequence of dehydration. A significant upsurge of aspartate amino transferase and creatine kinase activities was observed in all the animals. In conclusion, stress parameters were more influenced by transport and handling itself rather than by specific conditions related to different loading methods or crate position in the truck.

Key words: Rabbit, Loading method, Transport, Stress indicators, Welfare.

INTRODUCTION

Transportation is considered as a major stressor for farm animals and might have deleterious effects on the health, wellbeing, performance and, ultimately, product quality. Transport time has been considered one of the most critical points affecting animal welfare during the journey from farm to the abattoir (Marìa *et al*., 2006). However, transport involves several potential stressors including loading, unloading and penning in a new and unfamiliar environment and confinement with and without motion, vibrations, changes in temperature and humidity, inadequate ventilation and often deprivation of food and water. To date, even if stricter welfare rules for transport of animals have entered in force and even if the European Food Safety Authority has done numerous recommendations also for rabbit transport (EFSA, 2004), handling during loading represents, mainly for rabbits, a critical point that has been scarcely studied. Furthermore, the environmental conditions in the different points of the truck, usually without mechanical ventilation, represent, particularly during the summer, another condition that may influence the vehicle choice and safeguard of animal welfare.

The purpose of our investigation was to evaluate the effects of different loading methods and crate position on the truck on some stress indicators and on carcass and meat quality of commercial rabbits transported to the slaughterhouse during the hot season. Particularly, this paper will focus on data concerning weight losses and some blood parameters related to pre-slaughter stress and muscle metabolism.

MATERIALS AND METHODS

Animals and experimental design

A total of 192 animals, coming from the same farm and chosen from those that reached the end of their productive cycle (82 days old) were used. In order to allow time to collect and process analytical samples, the trial was carried out on 2 journeys in July. The journeys occurred in the morning (9.00 a.m.). The number of kilometres travelled was 123 and the mean transport time was 100 min (as net journey length), at an average speed of 73.8 km/h. The transport truck was owned by the abattoir and, as generally used in Italy, it was uncovered, 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 when it arrived on the farm: the rabbits were then collected and placed into the crates fixed in the truck. The number of rabbits transported on each journey was about 1500, filling the capacity of the truck. To evaluate the effects of crate position on the truck, at each session, 96 animals were distributed at random in 8 crates on the same side of the truck (12 animals per cage, 57.7 kg/m^2) as follows: 24 animals in 2 top front (TF) crates, 24 animals in 2 bottom front (BF) crates, 24 in 2 top rear (TR) crates and 24 in 2 bottom rear (BR) crates. In the middle of TF, BF, TR and BR crates four data-loggers (Extech instruments 42270) were then placed for temperature (T°) and relative humidity (%RH) control, with a record frequency of 1 min. To compare different loading methods one cage from each position was loaded in a smooth way (S) while the other one was loaded in a rough way (R). The S loading method consisted of carrying 12 rabbits from the farm crates into a wide trolley and then gently carrying each subject into the transport cage (loading time for 48 subjects: 12 min.). The R method, by contrast, consisted of carrying all 48 rabbits in the same trolley (as is usually done) and loading into four crates on the truck was executed hurriedly by the transport operator (loading time for 48 subjects: 4 min.).

Blood analysis

All the 192 rabbits (96 per journey) were individually weighed immediately before transport and again without delay at unloading in the slaughterhouse. To determine the effects of stress associated with the loading method or position in the truck, blood samples were collected from 40 male rabbits (20 per journey: 5 per truck position, 10 per loading method) 2 days before loading on the farm (basal level) and at slaughter during exsanguinations. Haematological parameters (packed cell volume - PCV, white blood cells – WBC; Neutrophils and Lymphocytes) were analysed with an Olympus AU400 autoanalyzer. EDTA plasma and serum were quickly obtained by centrifugation and aliquots were frozen (-20°C) for subsequent analysis. Plasma corticosterone concentration was determined by colorimetric detection using an ELISA kit (Neogen Corp., USA). Total protein (TP) and enzymatic activities of creatine kinase (CK), aspartate amino transferase (AST), alanine amino transferase (ALT) were analysed with an Olympus AU400 autoanalyzer.

Statistical Analysis

Data concerning temperature (T°) and relative humidity (%RH) in the truck were compared for the 4 different positions by an analysis of variance according to the GLM procedure of the SPSS version 13.0 statistical package (SPSS, 2006). Data concerning live weight and blood parameters before and after transport were analysed including the fixed effects of loading methods (R or S) and truck position (TF, BF, TR or BR). In order to determine the significances of differences between basal and after transport mean values, blood parameters were also analysed using a paired samples Student's t –test.

RESULTS AND DISCUSSION

Even if the 2 journeys took place in July in Central Italy, the mean outside temperature was not very warm, ranging from 15°C at loading to 25°C during unloading procedures at the slaughter house. Mean temperatures registered in the transport crates are presented in Table 1.

Table 1:Mean temperatures and relative humidity (in parenthesis minimal and maximal values) in the different crates on the truck during the two journeys

		TF Crates	BF Crates	TR Crates	BR Crates	P	MSE
Temperature	°C	25.16^{B} (22.1 - 29.7)	25.63^{B} (22.5 - 27.2)	27.21^{A} (24.7 - 29.0)	25.56^{B} (22.2 - 27.4)	0.000	0.15
Relative Humidity	%	41.05^{C} (31.9 - 48.1)	44.66^{B} (35.0 - 61.1)	37.14^{D} (26.9 - 55.9)	65.32^{A} (35.1 - 99.9)	0.000	0.69

Means with different letters on the same row differ significantly (A, B, C, D, P<0.01)

Few studies have focused on determining the appropriate thermal and humidity conditions for the transport of rabbits. EFSA (2004) estimates acceptable temperatures to be between 10 and 20°C. Extreme conditions, such as temperatures above 35°C or humidity below 55%, were found to be detrimental for rabbit welfare (Lebas *et al*., 1986). Thus, independently of their position, the rabbits in our trial experienced conditions higher than the upper limit recommended. This was particularly evident for the top-rear crates which showed the highest temperature and the lowest relative humidity (P<0.001) while the cages in the other three positions on the truck differed only in humidity, the bottom- rear one being markedly more humid.

Transport normally influences live weight; losses are probably related to transport time, a reduction in the gastrointestinal tract fill and losses from carcass components. Lambertini *et al*. (2006) found that losses increase significantly from 1.6 to 3.3% following journeys that lasted 1 to 4 hours. Live weight losses in the present study averaged 3% and were somewhat high if compared to the brief transport time (100 min.) (Table 2). No differences among the rabbits in the different crates were evident, even though the temperature and relative humidity were probably higher than the comfort zone in all crates.

Table 2: Effects of crates' position on the truck and loading method on live weight of rabbits

	TF Crates	BF Crates	TR Crates	BR Crates	P	Rough Loading	Smooth Loading	P	MSE
n. of rabbits	48	48	48	48		96	96		
Live weight before transport (g)	2421	2478	2457	2491	0.76	2474	2449	0.24	53.69
Live weight at slaughterhouse (g)	2341	2400	2370	2424	0.69	2391	2378	0.09	46.06
Weight loss (%)	3.27	3.12	3.51	2.68	0.13	3.38	2.91	0.18	0.53

Also the loading methods had no effects on weight losses during transport. Haematological parameters commonly used as indicators of stress during transport were also registered and are here presented as a contrast between pre and post transport results for each crate position in the truck or loading method (Tables 3 and 4).

Table 3: Effects of crates' position on the truck on haematological stress parameters of rabbits

		TF Crates				TR Crates			
		Before transport	At slaughter	P	MSE	Before transport	At slaughter	P	MSE
n. of samples		10	10			10	10		
WBC	($x10^3$/mcL)	12.65	12.47	0.871	0.99	13.28	12.73	0.531	0.78
PCV	(%)	40.13	39.10	0.306	0.83	39.20	41.00	0.127	0.86
Neutrophils	(%)	35.17^b	54.82^a	0.011	3.52	38.62^b	53.97^a	0.014	2.93
Lymphocytes	(%)	56.18^a	35.13^b	0.012	3.84	54.40^A	37.15^B	0.003	1.96
TP	(g/dL)	6.32	6.52	0.208	0.15	6.11^A	6.49^B	0.009	0.11
AST	(IU/L)	31.50	38.10	0.322	6.30	31.50	36.80	0.349	5.37
ALT	(IU/L)	33.70^b	39.20^a	0.019	1.93	27.80^b	33.50^a	0.037	2.32
CPK	(IU/L)	1025.00^B	3887.30^A	0.000	460.05	1180.40^B	3957.70^A	0.001	933.31
Corticosterone	(ng/ml)	11.42	13.69	0.754	7.01	11.60	27.99	0.119	9.52
		BF Crates				**BR Crates**			
		Before transport	At slaughter	P	MSE	Before transport	At slaughter	P	MSE
n. of samples		10	10			10	10		
WBC	($x10^3$/mcL)	11.64	11.52	0.940	1.52	12.48	13.61	0.409	1.18
PCV	(%)	40.81	39.14	0.238	1.28	41.78	39.35	0.118	0.51
Neutrophils	(%)	28.20^B	44.96^A	0.002	3.20	41.17^B	55.20^A	0.004	1.71
Lymphocytes	(%)	65.16^A	43.66^B	0.005	5.03	51.33^A	35.73^B	0.002	1.42
TP	(g/dL)	6.50	6.17	0.393	0.37	6.37	6.470	0.420	0.12
AST	(IU/L)	35.20	40.20	0.595	9.07	43.20	32.90	0.296	9.3
ALT	(IU/L)	30.70^b	34.30^a	0.033	1.43	33.40^b	36.40^a	0.049	1.63
CPK	(IU/L)	1007.00^B	3843.40^A	0.000	763.32	1413.40^B	3378.50^A	0.001	470.65
Corticosterone	(ng/ml)	8.98	22.36	0.196	9.58	6.38	26.20	0.098	10.74

Means with different letters on the same row differ significantly (a, b, P<0.05; A, B, P<0.01)

Transport and handling are often reported to evoke an increase in adrenal cortex responses and thus viewed as stressors (EFSA, 2004). Independently of the crate position in the truck, in our trial, corticosterone level increased from basal to post-slaughter levels in all animals; these differences, however, were not significant. Furthermore, no differences at post-transport sampling were found between the different crate positions. Liste *et al*. (2006) found that rabbits located in the top of the transport truck had lower corticosterone level 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 that of those housed in the bottom. The same authors related this finding to a possibly higher temperature in the bottom portion; our results do not confirm these findings. By contrast, plasma corticosterone levels increased significantly during transport following the rough loading method suggesting that it represents a severe stress for the animals. In the same way, Broom and Knowles (1989) found that plasma corticosterone concentration in hens at depopulation was three times as high after rough handling than after gentle handling. Similarly, an increase in WBC and neutrophilia, accompanied by a reduction in lymphocytes number and blastogenesis, were observed (Schaefer *et al.*, 1997). In our trial, leucocytosis was not present even if neutrophilia and lymphocitopenia were significant for all rabbits, independently of their position in the truck or the loading method. Transport and handling have been also observed to cause dehydration (Schaefer *et al.*, 1997) as a result of factors such as time off water, increased respiration rates and urinary water loss. Dehydration, particularly when transport conditions are combined with high transport temperatures, is associated with elevated packed cell volume (PCV) and plasma protein concentration. Liste *et al*. (2006) did not find any increase in PCV of rabbits transported at different levels in multi floor cage rolling stands. In our trial, PCV never differed significantly with respect to crate position or to loading method. Yet, rabbits transported in TR crates (with higher mean temperature) showed a significant augmentation of total protein (TP) level, probably as consequence of less comfort.

Similarly, rabbits loaded roughly showed a significant increase in TP. Finally a significant upsurge of AST and CK activities was observed in all the animals, independently of the crate position or loading method. Particularly, an increased CK activity can be interpreted as an index of cell muscle damage and muscle fatigue related to transport (EFSA, 2004). It is clear, however, that rough loading did not act as a major stressor with respect to normal transport and handling.

Table 4: Effects of loading method on haematological stress parameters of rabbits

		Rough Loading				Smooth Loading			
		Before transport	At slaughter	P	MSE	Before transport	At slaughter	P	MSE
n. of samples		20	20			20	20		
WBC	($x10^3$/mcL)	12.60	11.59	0.302	0.92	12.17	13.16	0.257	0.82
PCV	(%)	38.59	38.81	0.699	0.55	42.28	40.25	0.067	0.97
Neutrophils	(%)	35.71^B	50.02^A	0.001	2.85	33.59^B	52.05^A	0.000	1.17
Lymphocytes	(%)	56.74^A	40.23^B	0.001	2.95	59.30^A	37.55^B	0.000	2.73
TP	(g/dL)	6.32^b	6.55^a	0.043	0.10	6.32	6.27	0.801	0.19
AST	(IU/L)	30.40	35.40	0.140	3.25	40.30	38.60	0.812	7.03
ALT	(IU/L)	30.55^B	36.15^A	0.001	1.41	32.25^B	35.55^A	0.009	1.14
CPK	(IU/L)	958.10^B	3620.60^A	0.000	479.27	1354.80^B	3912.85^A	0.000	481.26
Corticosterone	(ng/ml)	9.70^b	28.86^a	0.017	7.31	9.50	16.26	0.226	5.41

Means with different letters on the same row differ significantly (a, b, $P<0.05$; A, B, $P<0.01$)

CONCLUSIONS

Even though they have been selectively bred for many years in captivity, several studies have shown that domesticated rabbits maintain a behavioural repertoire similar to their wild counterparts (Gunn and Morton, 1995). Accordingly, rough handling should procure more stress in rabbits than in other animals thus influencing slaughter performances. Furthermore, even if rabbits tolerate higher climatic stress than do large mammals, heat stress related to different positions in the transport truck could also negatively influence their welfare. However, the results obtained in the present study indicate that stress parameters were more influenced by transport and handling itself than by specific conditions related to different loading methods or crate position on the truck.

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