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Effects of loading methods on rabbit welfare and meat quality

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ABSTRACT

The effects of different loading methods on the welfare, carcass characteristics and meat quality traits of hybrid commercial rabbits were investigated. 384 male rabbits, 82 days old, were transported from the farm to the slaughterhouse. At the farm, 192 rabbits were loaded onto the truck smoothly (S) and 192 rabbits were loaded roughly (R). The S loading method consisted of carefully placing each rabbit into the transport crates. In the R method, the loading was hurriedly and carelessly executed by the transport operator, throwing each animal into the crates fixed on the truck.

Live weight before and after transport as well as slaughter data were recorded for each rabbit, and a subset of 80 carcasses were evaluated for meat quality. Blood samples from 80 rabbits were analysed for haematological and biochemical parameters. A significant neutrophilia ($P < 0.001$), lymphocytopaenia ($P < 0.001$) and an increase 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, independent of the loading method. A twofold increase in serum corticosterone concentration (6.23 vs. 14.88 ng/mL; $P = 0.001$) was observed in all rabbits following transport. Results suggest that the stress parameters analysed were more influenced by transport and handling itself rather than by the different loading methods. The results showed that there was no adverse effect of loading method on carcass traits. Furthermore, the stress condition evidenced by haematological and biochemical parameters prior to slaughter did not affect meat quality.

1. Introduction

It is widely accepted that journey length is an aspect that may affect animal welfare during transport from the farm to the slaughterhouse (Gosàlvez, Averò, Valdelvira, & Herranz, 2006; Grandin, 2000). Transportation not only affects animal welfare, but it can also have a negative influence on meat quality, and it may cause economic losses (Jolley, 1990; María et al., 2006; Perez et al., 2002; Warriss, 1990, 2000). In addition to transport time, other factors can influence animal welfare during road transport and subsequent meat quality, such as loading and unloading (Buil, María, Villarroel, Liste, & López, 2004; Nanni Costa, Lo Fiego, Dall'Olio, Davoli, & Russo, 1999), stocking density, weather conditions (temperature, air velocity and humidity), vehicle characteristics, food and water deprivation or mixing animals from different groups (Verga, Luzi, Petracci, & Cavani, 2009). From both animal welfare and economic points of view, it is necessary to control and minimise stress-inducing factors during transport and before slaughter (Van de Water, Verjans, & Geers, 2003). At the present time, even though strict welfare rules for transport of animals are enforced and the European Food Safety Authority (EFSA, 2004) has distributed numerous recommendations about rabbit transport, handling during loading is a critical point that has been little studied, especially for rabbits. Vignola, Giammarco, Mazzone, Angelozzi, and Lambertini (2008) found that, in rabbits transported to the abattoir, stress parameters were more influenced by transport and handling than by specific conditions related to different loading methods.

Due to the lack of a specific regulation for appropriate management of the pre-slaughter phase in rabbits, the loading method could represent a critical point that may lead to negative consequences on both animal welfare and product quality.

In other livestock, loading methods seem to have an important role in transport stress.

The complete set of transport events, in particular the loading and unloading phases, have been reported to determine stress and affect meat quality in calves (Van de Water et al., 2003). In heavy pig commercial transport, Nanni Costa et al. (1999) showed that, although the loading method could play an important role in increasing the level of stress, with short journey times (less than about 1 h), loading by ramp or lift had negligible effects on meat and dry-cured ham quality. The duration of crating (Kannan et al., 1997) and the method of crating (Duncan, 1989) are factors that most likely cause physical injuries in broilers and can also influence their stress response.

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Until now, rabbit welfare during transport has not been studied as widely as in other livestock, and there is limited evidence about the effects of pre-slaughter stress factors on rabbit physiology and product quality.

The aim of the present study was to evaluate whether different loading methods on the truck (smooth vs. rough loading) influenced some stress parameters, carcass traits and the meat quality of commercial rabbits during transport to the slaughterhouse.

2. Materials and methods

2.1. Animals and experimental design

Animal handling and transport followed the recommendations of Regulation 1/2005/EC.

To evaluate the effects of loading methods, a total of 384 male rabbits 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). The trial was started from April, and the journeys took place in four consecutive sessions: one every 2 weeks.

The 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 ($98 \times 52 \times 24$ cm, length × width × height) provided with loading doors were already on the truck.

For each journey, 96 rabbits were placed into 8 crates fixed on the same side of the truck (12 animals per cage, 57.7 kg/m^2). The experimental rabbits were placed always in the same experimental crates which were located in the same position on the truck. The number of rabbits transported for each journey was about 1500, which filled the capacity of the truck. The journey time was 100 min Moreover, for each journey, the same lorry driven by the same operator was used.

In particular, to compare two different loading methods, four cages were loaded smoothly (S), while the others were loaded roughly (R). The S loading method consisted of moving rabbits from the farm cages to the lorry using four wide trolleys (12 animals/trolley) and gently placing each rabbit into the transport crates; the loading time for 48 subjects was 12 min. By contrast, the R method consisted of carrying all 48 rabbits in the same trolley and the loading was executed hurriedly and carelessly by the transport operator by throwing the rabbits into the crates on the truck; the loading time for 48 subjects was 4 min.

2.2. Blood analysis and stress measurements

In order to assess pre-slaughter stress, blood samples from 80 rabbits (20 animals per treatment per journey) were collected 2 days before each transport session and at bleeding in the slaughterhouse. The blood (5 mL) from each rabbit was taken from the central ear vein with a 5 mL syringe and 22-ga needle and placed into an EDTA tube in a serum separator tube (Terumo Venoject, Belgium). Samples were kept refrigerated until arrival at the laboratory, where they were processed immediately. Haematological parameters, including 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 *g* for 10 min, divided in two aliquots and immediately frozen (−80 °C). One aliquot was used to analyse for glucose, total protein (TP), sodium (Na), potassium (K) and activities of creatine kinase (CK), lactate dehydrogenase (LDH), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) with an Olympus AU 400 auto-analyser.

Serum biochemical data were used to calculate the equivalent osmolality (OsmE, Eq. (1)) and Total osmolality (OsmT, Eq. (2)), as follows:

$$2(\mathrm{Na}+\mathrm{K})+\mathrm{GLU}/18=\mathrm{OsmE}(\mathrm{mOsm/L}) \quad (1)$$

$$2(\mathrm{Na}+\mathrm{K})+\mathrm{GLU}/18=\mathrm{BUN}/2.8=\mathrm{Osm_T}(\mathrm{mOsm}/L) \quad (2)$$

where Na^+ is sodium (mmol/L), K^+ is potassium (mmol/L), GLU is glucose (mg/dL) and BUN is blood urea nitrogen (mg/dL) (Schermerhorn & Barr, 2006).

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 kit supplier recommendations were followed. Final absorbance was measured in a microplate reader (DV 990 BV6, GDV S.r.l., Rome, Italy).

2.3. Carcass traits and meat quality

All the rabbits were individually weighed before transport (live weight before transport – LWBT) and again immediately after arrival at the abattoir (slaughter live weight – SLW). Slaughtering procedures began without delay after weighing.

Rabbits were electrical stunned (105 VAC, 240 Hz, 1.1 A, 2 s), hung upside down on a rail and slaughtered by severance of the jugular veins. The animals were then processed following standard industrial techniques. For each one, the weight of the full gastrointestinal tract (including stomach, caecum, intestinal contents and urogenital tract with empty urinary bladder) and of the hot and chilled (4 °C for 1 h) carcass (inclusive of the head, kidneys, liver, heart, lungs, scapular and perirenal fat) were recorded, as outlined by Blasco and Ouhayoun (1993).

On 80 randomly chosen carcasses (20 for each journey, half from the smooth loaded group and half from the rough-loaded group) pH_1 was measured in situ on the *Longissimus dorsi* muscle of the right side between the 6th and the 7th lumbar vertebra. This was conducted 15 min. after slaughter, before transfer into the chilling room, using a penetrating electrode (Double Pore Slim, Hamilton Company, Bonaduz, Switzerland) adapted to a portable pH meter (HD 8705, Delta Ohm, Padova, Italy) as outlined by Ouhayoun and Dalle Zotte (1996). The pH was adjusted for meat temperature.

During the first 24 h post-mortem, the carcasses were stored in a ventilated cold room at 4 °C, after which the ultimate pH (pH_u) was measured on the same muscle (Ouhayoun & Lebas, 1995). Meat colour was measured using a Minolta chromameter (Minolta CR-300, Tokyo, Japan) with a D65 illuminant and an 8-mm aperture in the measuring head. Colour measurements were reported in the CIE $L^*a^*b^*$ system in terms of lightness (L^*), chroma [$C^* = (a^2 + b^2)^{0.5}$] and hue [$H^\circ = \arctan(b/a)$].

Colour measurements were taken across the same cross section of muscle on the left side, avoiding areas of connective tissue or intramuscular fat.

Drip loss and cooking loss were determined on meat samples about 20 g each and roughly cubic in shape from *Longissimus dorsi* muscle, as suggested by Honikel (1998). Drip loss was measured in duplicate samples attached to plastic netting and suspended in an inflated plastic bag for 24 h at 2 °C. Drip loss was the difference in weight pre- and post-suspension and was expressed as a percentage of the initial weight.

Samples for cooking loss determination were weighed, placed in thin-walled plastic bags and cooked in a continuously boiling water-bath, with the bag opening extended above the water surface until the internal temperature reached 75 °C. Samples were then removed from the water-bath, cooled in running water and held at

2 °C until equilibrated. The meat was then taken from the bag, blotted dry and weighed. Cooking loss was the difference in weight between the pre-cooked and blotted dry post-cooked weights, and was expressed as a percentage of the pre-cooked weight.

2.4. Statistical design and analysis

Data were tested for normal distribution using the K S – *Liliefors test*. Blood parameters obtained before and after transport were compared using a paired samples Student's *t*-test. The same results were transformed into the ratio between basal and post-transport values. These data and those concerning live weight were analysed according to the GLM procedure of the SPSS version 13.0 statistical package (SPSS, 2006), including the fixed effects of loading methods (R or S) and transport session. The effect of the transport sessions is not reported because it was not significant. When the analysis of variance *F*-test was significant ($P < 0.05$), differences among means were compared using the Student–Newman–Keuls test. The Pearson correlation ($P < 0.01$) was applied between variables in order to establish whether welfare parameters were related to meat quality parameters. Results are presented as treatment means, and variance is expressed as the standard error.

3. Results and discussion

3.1. Effect of loading method and transport on blood measurements

The mean values of haematological and biochemical variables in relation to transport and loading methods (Tables 1–4) show that the variables considered were significantly influenced by transport itself, rather than by loading method.

Transport has been observed to cause dehydration (Schaefer, Jones, & Stanley, 1997) as a result of factors such as time without water, increased respiration rates and urinary water loss. Dehydration is associated with both elevated packed cell volume (PCV) and plasma protein concentration (Broom, 2003; Schaefer et al., 1997).

By contrast, in this study, PCV after transport did not differ from values detected at the farm, probably because of the short duration of the transport. This agrees with Liste et al. (2008), who did not find any variation of PCV in commercial rabbits transported to the abattoir.

Haematological data showed that differential leukocyte count was significantly influenced by transport. Further, the white blood cell (WBC) count was significantly influenced by transport, as evidenced by the increase observed in all rabbits after transport. Leukocytosis in acutely stressed animals is caused by the endogenous release of corticosteroids or epinephrine.

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

	Sampling time		*P* Values	St. Err.
	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

Table 3
Effects of loading methods on haematological parameters of rabbits[a].

	Loading method		*P* Values	St. err.
	Rough (R)	Smooth (S)		
Number of samples	40	40		
Red blood cells	0.98	0.98	0.752	0.008
Haemoglobin	0.98	1.49	0.144	0.167
Packed cell volume	0.99	1.00	0.665	0.009
White blood cells	1.44	1.29	0.201	0.062
Neutrophils	1.32	1.23	0.276	0.042
Lymphocytes	0.84	0.86	0.764	0.043
Neutrophil:lymphocyte ratio	1.87	1.64	0.312	0.110

[a] Results expressed as a ratio between basal and post-transport values.

Table 4
Effects of loading methods on biochemical parameters of rabbits[a].

	Loading method		*P* Values	St. err.
	Rough (R)	Smooth (S)		
Number of samples	40	40		
Aspartate aminotransferase (AST)	1.75	1.62	0.482	0.090
Alanine transferase (ALT)	1.25	1.19	0.151	0.022
Creatine phosphokinase (CK)	4.28	3.75	0.313	0.258
Lactate dehydrogenase (LDH)	2.08	2.04	0.906	0.181
Glucose	1.19	1.14	0.309	0.022
Total protein	1.05	1.03	0.455	0.012
Albumin	1.04	1.06	0.545	0.014
Na	1.08	1.09	0.687	0.014
K	1.44	1.37	0.198	0.030
Urea	1.02	1.00	0.635	0.147
OsmT	1.09	1.09	0.889	0.012
OsmE	1.09	1.10	0.841	0.012
Corticosterone	6.89	5.90	0.722	1.386

[a] Results expressed as a ratio between basal and post-transport values.

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

	Sampling time		*P* Values	St. err.
	Before transport	After transport		
Number of samples	80	80		
Aspartate aminotransferase (AST) (UI/L)	25.39	32.58	0.001	2.096
Alanine transferase (ALT) (UI/L)	31.84	38.04	<0.001	0.641
Creatine phosphokinase (CK) (UI/L)	885.86	2905.76	<0.001	195.837
Lactate dehydrogenase (LDH) (UI/L)	229.69	234.96	0.882	35.312
Glucose (mg/dL)	139.98	160.64	<0.001	3.497
Total protein (g/dL)	5.80	6.01	0.004	0.072
Albumin (g/dL)	3.71	3.89	0.001	0.054
Na (mmol/L)	139.43	149.56	<0.001	1.176
K (mmol/L)	5.54	7.54	<0.001	0.151
Urea (mmol/L)	32.40	32.66	0.628	0.539
OsmT (mOsm/L)	309.28	334.79	<0.001	2.426
OsmE (mOsm/L)	297.71	323.13	<0.001	2.384
Corticosterone (ng/mL)	6.23	14.88	0.001	2.465

Another useful measure of the sustained effect of stress is the neutrophils/lymphocytes (N:L) ratio, which increased due to transport stress. Schaefer et al. (1997) and Kegley, Spears, and Brown (1997) also observed an increase in the N:L ratio with transportation stress in cattle. The N:L ratio has been used as a reliable index of stress in birds (Minka, Ayo, & Fayomi, 2004; Scope, Filip, Gabler, & Resch, 2002) and other livestock (Kannan et al., 2000; Stull & Rodiek, 2000).

In the present study, a significant increase in the N/L ratio and in the neutrophils percentage and a decrease in lymphocyte percentage were observed in all transported rabbits. These results indicate that, for rabbits, even a short transport significantly influences haematological parameters that are usually used as stress indicators.

Independent of the loading method, a significant increase in serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) and creatine kinase (CK) activities was observed in all rabbits. Lactate dehydrogenase activity was not influenced by transport or loading method, as shown in Tables 2 and 4. The increase in AST, ALT and CK activities could be interpreted as an index of cell muscle damage and muscle fatigue (Payne & Payne, 1987). In birds, muscle enzyme activity increases during capture and handling operations because of increased muscle cell permeability or muscle cell damage (Duncan & Prasse, 1986). CK is the most sensitive enzyme that shows muscle damage and even intense muscle activity.

In particular, increased CK activity can be interpreted as an index of muscle fatigue related to transport (EFSA, 2004). In goats, Kannan, Kouakou, Terrill, and Gelaye (2003) found that vigorous physical activity, such as herding, loading and unloading procedures, were more important than transportation itself in determining plasma CK activity. Regarding LDH, treatment during transport did not influence this parameter, probably because rabbits did not suffer severe enough muscle damage to justify an increase of enzymatic activity.

A significant increase in serum glucose concentration following transport, independent of the loading method, was found. This was probably due to glycogenolysis associated with the increase in catecholamine and glucocorticoids, which are released during stressful conditions, as reported by Shaw and Tume (1992). A rapid increase in plasma glucose concentration has been seen in goats after a short transport (Kannan et al., 2003). These authors also found an increase in plasma glucose concentration during the immediate pre-slaughter period that directly correlated to that of plasma cortisol. On the contrary, Liste et al. (2006) recorded a significant decrease in plasma glucose and high lactate levels in rabbits transported to the abattoir and showed that journey length did not affect plasma glucose concentration. They suggested that this was probably because of an increase in glucose consumption, resulting from the stress of transport. According to Knowles et al. (1995), sheep 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 inducing a mobilisation of body energy reserves. Kannan et al. (2000) also noted that as a response to stress, elevation of glucose concentration was preceded by an elevation of the cortisol concentration.

Total serum proteins and serum osmolality can both be used as measures of dehydration and the stress condition (EFSA, 2004). Usually, dehydration is associated with an increase in packed cell volume (PCV) and plasma protein concentration. As previously stated, PCV was not influenced by transport or loading method. Furthermore, total protein, albumin and osmolality increased significantly in all rabbits, independent of the loading method. These results suggest an activation of the stress response system and moderate dehydration caused by transport: it is evidenced also by the increasing Na and K serum concentrations.

The events occurring prior to slaughter lead to physical stress as well as psychological stress. Animals respond physiologically to the perception of stressful stimuli through a rapid cascade of endocrine secretions within the hypothalamic–pituitary–adrenal (HPA) axis (Sapolsky, Romero, & Munck, 2000). This response involves 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 (Creel, Creel, & Monfort, 1996; Romero & Wikelski, 2001). Corticosterone is the major adrenal glucocorticoid secreted by the European rabbit (Szeto et al., 2004). Serum corticosterone increases in all rabbits due to the activation of the stress response system. In particular, a twofold increase in serum corticosterone was observed in all rabbits following transport. In the present study, corticosterone serum concentration seems to be more influenced by transport than by loading method.

These results agreed with those of Duncan (1989), who found that broilers crated and transported on a vehicle for 40 min had higher plasma corticosterone concentrations than broilers that were crated and loaded onto the vehicle but not transported. Further, Cashman, Nicol, and Jones (1989) reported that fear levels in broilers were mainly determined by transportation and not just by catching and loading. By contrast, in cattle, Pettiford et al. (2008) and Warriss et al. (1995) found that loading procedures and the initial stages of transport are the most stressful events of the pre-slaughter phase; after this period, animals adapt to the conditions.

The results demonstrate that rabbits undergo stress during transfer from the farm to slaughterhouse. However, the different loading methods did not exert significant differences in the stress indicators.

3.2. Live weight and carcass characteristics

Table 5 shows the effects of loading method on live weight and slaughter data. No skin damage and no deaths were seen after transport.

Table 5
Effect of loading methods on live weight and slaughter data of rabbits (mean values).

	Loading method		*P* Values	St. err.
	Rough (R)	Smooth (S)		
Number of samples	192	192		
Live weight before transport (LWBT), g	2606	2582	0.922	25.565
Slaughter live weight (SLW), g	2528	2510	0.714	24.984
Weight losses, %	3.00	2.78	0.162	0.184
Hot carcass weight (HCW), g	1535	1530	0.784	14.534
Chilling losses, %	1.96	1.85	0.501	0.242
Dressing percentage (LWBT), %	59.0	59.3	0.711	0.270
Dressing percentage (SLW), %	60.8	61.0	0.808	0.299
Full gastrointestinal tract weight (LWBT), %	16.7	16.7	0.592	0.215
Full gastrointestinal tract weight (SLW), %	17.2	17.2	0.745	0.219

The LWBT was consistent with the weight usually reached by rabbits at the end of their productive cycle. The different treatment did not significantly affect either SLW or losses. Live weight losses averaged 2.89%, and were somewhat high given the brief transport time (100 min).

Loading at the farm involves considerable stress for rabbits that are forced to move from the fattening unit to the truck interior. Pre-slaughter handling and transport may increase body weight loss during transport (Masoero, Riccioni, Bergoglio, & Napolitano, 1992) from 1.4% to 4.6% as the transport duration increases from 1–7 h (Luzi, Heinzl, Crimella, & Verga, 1994).

Transport duration normally determines live weight losses. In a short journey, the live weight loss is mainly related to the reduction in the gastrointestinal tract fill. However, the weight reduction can also depend on other losses, principally due to dehydration, which are observed only on more prolonged journeys. In our trial the short transport time determined the lack of difference in hot carcass weight between the groups (1535 vs. 1530 g, R vs. S; $P = 0.784$). Lambertini, Vignola, Badiani, Zaghini, and Formigoni (2006) found that losses increase significantly from 1.6 to 3.3% following journeys that lasted 1–4 h and that they were about 2% when rabbits were transported for 2 h. Luzi et al. (1994) and Trocino, Xiccato, Queaque, and Sartori (2003) reported a similar trend for a 2 h journey.

The full gastrointestinal tract weight accounted for 16.7% of LWBT, which is in good agreement with Trocino et al. (2003) and slightly lower than found by Lambertini et al. (2006) after a short transport time (2 h). In the present study, loading method did not affect this parameter.

Likewise, the dressing percentages were not influenced by the experimental treatment. Values of 59.14% and 60.91% were found for yield calculated on LWBT and SLW, respectively. These results were in agreement with the findings of Lambertini et al. (2006) for rabbits slaughtered after 2 h of transport, whilst other authors have reported lower values (Dalle Zotte, Rizzi, & Riovanto, 2008; Hernàndez, Ariño, Grimal, & Blasco, 2006; Petracci, Bianchi, & Cavani, 2008).

In addition, the chilling loss from the carcasses, which mainly depends on water losses due to evaporation (Jolley, Lopes, Dransfield, & Perry, 1983), was not influenced by treatment.

The different loading methods had no significant effect on all the parameters. It is possible that the stress experienced by rabbits during transfer on the truck, even in the rough-loaded one, was not sufficient to affect slaughter data.

There are no data concerning the effect of loading methods on slaughter parameters in rabbits. In other species, loading method seems to have an important influence on welfare but not on slaughter data. Kuchenmeister, Kuhn, and Ender (2005), by subjecting pigs to "gentle" handling compared to the use of nose snare and electric goad, found no significant differences in live and carcass weights or lean meat yield. Hambrecht et al. (2005) found no differences in carcass characteristics of pigs subjected to different stressful conditions before transport to the slaughterhouse. Moreover, in pigs transported to the abattoir, Nanni Costa, Lo Fiego, Dall'Olio, Davoli, and Russo (2002) found that the loading method had no significant effect on carcass traits.

3.3. Meat quality

Meat quality data are shown in Table 6. Muscle pH_u is an important determinant of meat quality (Watanabe, Daly, & Devine, 1996), and is related to the rate of glycogen breakdown and liberation of lactate pre- and post-slaughter. Normally, acute stress caused by handling and transport depletes the muscular glycogen reserves because of the secretion of catecholamine and usually increasing pH_u values cause darker meat colour (Warriss, Brown, Adams, & Corlett, 1994).

Table 6
Effect of loading methods on pH and meat quality parameters of rabbits (mean values).

	Loading method		*P* Values	St. err.
	Rough (R)	Smooth (S)		
Number of samples	40	40		
pH_1	6.88	6.88	0.909	0.022
pH_u	5.79	5.81	0.697	0.023
$pH_1 - pH_u$	1.09	1.07	0.705	0.031
L^*	57.55	57.29	0.789	0.469
A^*	2.65	2.61	0.863	0.120
B^*	1.64	1.75	0.657	0.123
C^*	3.22	3.24	0.926	0.145
H°	28.95	33.27	0.220	1.750
Cooking loss, %	19.76	17.92	0.008	0.338
Drip loss, %	3.10	3.21	0.722	0.155

In our study, pH_u values were not significantly affected by loading method (5.79 vs. 5.81 for R and S, respectively; $P = 0.697$) and agreed with those reported previously (Dal Bosco, Castellini, & Bernardini, 1997; Lambertini et al., 2006; María et al., 2006) for short transport (2 h).

On the contrary, Lambertini et al. (2006) reported higher pH_u values (6.01) and lower pH fall when rabbits were subjected to long journeys (4 h), while Trocino et al. (2003) observed no increase in pH_u after a transport of the same length. María, Villarroel, Sañudo, Olleta, and Gebresenbet (2003) reported no significant relationship between meat pH_u and journey length in animals transported for less than 6 h.

In the present study, both groups had a mean pH_u lower than 6.0, which is considered essential for good product quality (Mach, Bach, Velarde, & Devant, 2008; Terlouw, 2005).

The rough loading method did not affect muscle acidification, even if it caused a more intense stress response pre-slaughter and probably greater glycogen consumption. Dalle Zotte (2002) reported that pre-slaughter treatments do not lead to anomalies, such as PSE or DFD in rabbits. Commonly, the meat from transported rabbits has a higher pH_u and, as a consequence, has higher WHC (water-holding capacity) and appears to be darker, less colourful and more tender by instrumental or sensorial evaluation (Ouhayoun & Lebas, 1995). On the contrary, short-term transportation should improve the sensory qualities of rabbit meat, making it more tender and juicy (Masoero et al., 1992).

In other livestock, various stress factors have been reported to affect meat pH: time and handling during transportation from farm to slaughterhouse (Arthington, Eichert, Kunkle, & Martin, 2003), time at the slaughterhouse (Warriss, 2003), climatic factors (Silva, Patarata, & Martins, 1999), social disruption (Hambrecht et al., 2005), and the novelty of the pre-slaughter environment (Hambrecht et al., 2005; Mounier, Dubroeucq, Andanson, & Veissier, 2006). Although several authors suggest that meat pH is affected by stress factors, there is little information concerning the effect of the interaction between these factors and meat pH as well as the impact of each factor on meat pH variability (Mach et al., 2008).

The present study, found no evidence of a link between blood corticosterone and meat pH.

It is possible that the amount of stress caused by the rough loading method was not enough to influence the muscle glycogen content and meat acidification process.

Furthermore, Mounier et al. (2006) stated that the measurement of pH_u could be an insensitive measure of physical exhaustion and physiological pre-slaughter stress. The physiological responses of animals during the whole transfer process would be

necessary to understand which phase prior to slaughter is the most stressful and the most important factor affecting meat pH.

Ante-mortem stress can affect meat texture and colour (Dalle Zotte, 2002; Masoero et al., 1992) but little is known about the effects of pre-slaughter stress on the colour of rabbit meat (Liste et al., 2009). Jolley (1990) reported that transport time decreases lightness and colour saturation, which makes meat look darker, but with no detrimental effect on quality.

In this study, meat colour averaged 57.42, 3.23, 31.11 for L^*, C^* and H°, respectively, and ranged within the normal quality range indicated by Dal Bosco et al. (1997), Lambertini et al. (2006) and María et al. (2006) for short distance transport. However, the loading method did not affect meat colour, and no differences were recorded for this parameter between the treatments.

Warriss et al. (1994) found darker meat with higher colour saturation from pigs subjected to stress before slaughter. Ruiz-De-La-Torre et al. (2001) also compared different transport conditions (smooth transport vs. rough transport) and found significantly higher pH_u and a^* values in meat from sheep that were roughly transported.

Muscles from rough-loaded rabbits had significantly higher cooking loss than those from smooth loaded ones (19.76 vs. 17.92%; $P < 0.01$). As stated by Dalle Zotte, Parigi Bini, Xiccato, and Simionato (1995), water losses in meat increase as pH_u decreases and the same authors showed that cooking loss is negatively correlated to pH_u.

The present study did not confirm this observation. In fact, the rough-loaded rabbits had higher cooking losses that were not related to any pH_u modification. It was possible that the severe stress caused by the rough loading method and the cell damage evidenced by the significant increase in AST in this group, caused higher myofibrillar shrinkage when the meat was cooked. The loading method did not affect meat drip losses. However, other studies have found that an increase in ultimate pH increases WHC, which, in turn, decreases water losses during cooking and increases the cutting force (Jolley, 1990; Trocino et al., 2003). In cattle, Warner, Ferguson, Cottrell, & Knee (2007) found that cooking losses increased, even though the pH decline rate and pH_u were not affected. A possible explanation for the loss in water-holding capacity was that the 'stress' treatment caused a shift in the distribution of ions in the muscles as ions passed into the plasma pre-slaughter. This would have decreased the osmolarity of the fluid in the muscle at slaughter. This diffusion of ions out of the muscles would continue post-slaughter, with more ions appearing in the fluid expressed from meat during storage. Release and diffusion of free ions across the sarcolemma contribute to the degree of swelling or shrinking of the myofibrillar lattice, and therefore, they affect the space available to hold water. This interpretation could also explain the cooking loss findings.

To determine the effect of stress on meat quality, a correlation between muscle enzymatic activity and meat pH was performed.

CK activity was weakly correlated with pH_u values, for both rough-loaded ($r = 0.38$, $P < 0.05$) and smooth-loaded rabbits ($r = 0.35$, $P < 0.05$). A better relationship was found between LDH and pH_u (R group: $r = 0.41$, $P < 0.01$; S group: $r = 0.44$, $P < 0.01$). No significant correlation was found with other meat quality parameters.

These results suggest that there is a relationship between muscle cell damage and muscle pH.

The results agree with the findings of Warriss, Brown, Edwards, and Knowles (1998) for pigs. Although these authors did not record a linear relationship, the distributions of data points showed that pigs producing meat with higher pH_u tended to have higher circulating levels of cortisol, lactate and CK. On the contrary, other authors showed that CK and LDH activities were negatively correlated with muscle pH_u in pigs transported to slaughter (Perez et al., 2002).

Based on our findings, loading methods did not have a significant effect on meat quality and confirm that the threshold for stress to have an effect on meat quality may be higher than the threshold to have an effect on welfare indicators (Liste et al., 2006).

4. Conclusions

The results showed that the analysed stress parameters were more influenced by transport and handling than by specific conditions related to different loading methods onto the truck.

The loading method did not negatively affect carcass and meat quality traits. The rough loading method could be an additional stress for animals that may be negatively affected by, in particular, environmental transport conditions, such as high temperatures during the journey.

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