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THE EFFECT OF TRANSPORT TIME, SEASON AND POSITION ON THE TRUCK ON STRESS RESPONSE IN RABBITS

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Abstract: The present study analyzed the effect of transport time, season and position on the truck on physiological stress response of commercial rabbits in Aragón (Spain). A total of 156 animals were sampled in a 2×2×3 factorial design testing two transport times: short, 1 hour (1hT) and long, 7 hours (7hT), in two different seasons: hot, during summer (HT) and cold during winter (CT), and three different positions on the truck: upper, middle or lower decks in multi-floor cages on rolling stands (MFRS-top, MFRS-middle and MFRS-bottom). Three replicates were performed per treatment. Blood samples were taken at sticking during slaughter to compare hematocrite, corticosterone, glucose, lactate and Creatine Kinase (CK) levels as well as the ultimate pH of the carcass (pH24). Corticosterone and CK levels were highest in 1hT rabbits. With respect to season, colder temperatures increased corticosterone, while warmer temperatures increased CK (P<0.001). Regarding position on the truck, MFRS-middle and bottom rabbits had higher levels of glucose, corticosterone and CK. The pH24 values were within normal ranges for all treatments but slightly higher for animals transported in winter. In general, transport time and season were significant stressors for commercial rabbits, due to the effects on their physiological states. Position on the truck seems to have an effect on stress response to transport in rabbits. However, pH24, which is considered one of the main parameters of welfare measurements, was not affected by transport time or position on the truck.

Key words: rabbit, transport, season, animal welfare, blood samples.

INTRODUCTION

In addition to breeding, rearing, and fattening, meat production systems include transport, lairage, slaughter and dressing at the abattoir. This production chain has several critical points, such as the transport from farm to abattoir, which, although brief involve potential risk (Buil *et al*., 2004). Poor handling during transport and slaughter can cancel out good results during the growing and fattening phases (Villarroel *et al*., 2001). Many circumstances involved in this process can induce stress in animals and have negative effects on animal welfare (Jolley, 1990).

Over the last few years European countries have taken an increased interest in animal welfare, housing systems and husbandry. Transport may be considered as an extension of handling on the farm, however

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this housing is now in movement through changing environments that challenge the adaptive mechanisms of the animal. If these environmental changes require extreme adjustments for adaptation, they are considered stressful for animals.

In general, there is little information available on the effect of transport on the welfare of commercial rabbits. However, it is known to be one of the main causes of stress, especially in Mediterranean countries with high summer temperatures (Canali *et al*., 2000).

In Spain, 100 million commercial rabbits are transported and slaughtered per year. Since lorries travel on roads and through towns, the process is visible to consumers. Correct handling and reduction of associated risks during this period will increase the quality of the final product, as well as improve the product's image (Lambertini *et al*., 2006). The combination of the two concepts, product quality and animal welfare, will help to increase the success of establishing handling practises that improve animal welfare.

The objective of this study was to evaluate the effect of transport time from farm to slaughterhouse, in addition to season and position on the truck, on some physiological indicators of stress that can affect the welfare of commercial rabbits.

MATERIAL AND METHODS

Animals and Treatments

The study was carried out in the Autonomous Community of Aragon (northern Spain) with two commercial farms that produced rabbits with similar handling and feeding methods. Commercial hybrid rabbits were fed *ad libitum* with commercial concentrate from weaning at 30-35 days of age and slaughtered at 2 months of age (live weight 2300 g). The animals were all slaughtered at an EU-approved abattoir in Villanueva de Gállego (Zaragoza) by electrical stunning and sticking. Two transport periods (1h, 1hT and 7h, 7hT) and two seasons (summer, HT and winter, CT) were analyzed. The study was carried out in June-July 2003 (HT) and December 2003-January 2004 (CT). The position on the multi-floor cages on rolling stands (MFRS) was also included as a variable, considering the top (4 animals), middle (5 animals) and bottom (4 animals) positions in each treatment. Three replicates were performed for each treatment and 156 animals were analyzed in total, 13 for each of the twelve treatments (13 animals×2 transports×2 seasons×3 replicates). To determine the basal levels of the parameters analyzed, blood samples were taken from a control group of 30 rabbits randomly selected from both farms and subjected to the same breeding conditions. This group of rabbits was not submitted to any transportation treatment so as to establish physiological blood basal values.

Transportation

The truck, provided by the abattoir, had a rigid chassis, spring-suspension but had no climate control. The roof was made of fibreglass and the side walls were open bars with retractable curtains. The truck had a hydraulic platform for loading and unloading. There was a 25 cm gap between the upper part of the MFRS and the roof and the bottom part of the MFRS and the floor to allow for ventilation. The driver left a space free of cages in the front and back areas of the truck to help air circulation. The lorry was 6.20×2.50×1.85 m (long×wide×high), and the animals travelled in 20 MFRS (150×120×60 cm) with 12 cages each. Total capacity was 2400 rabbits per journey. The stocking density during the journey was approximately 10 rabbits per cage, equivalent to 360 cm^2 per animal (cage size = 25×60×60 cm). This space allowance is almost half the recommended 600 cm^2 per animal given in the latest EFSA report on transportation of rabbits (EFSA, 2004) but is similar to normal commercial practice. Average temperature and humidity was recorded every 5 min using data loggers (Testo®) located at the mid section of the MFRS during transport.

The 7hT treatment started at 5:00 am from Farm One and the 1hT treatment at 11:00 am from Farm Two. In both cases, animals were unloaded at the abattoir at midday. On arrival, a lairage period of 3 h was observed before the animals were slaughtered. In summer, animals were showered for a 30 sec period in the waiting room (before lairage), following normal abattoir practice. The animals remained in their assigned MFRS during lairage until slaughter, when they were removed from the transport cages and stunned electrically using a V-shaped metal electrode.

Parameters analyzed

To evaluate physiological response to stress in rabbits, blood samples were collected at exsanguinations (two 10 mL tubes per animal, with and without anticoagulant, EDTA-K_3). Before arriving at the laboratory, samples were kept on ice for a maximum of 2 h. Plasma EDTA and serum samples were centrifuged at 3000 rpm for 10 min and aliquots were frozen and stored at -30ºC. Hematocrite values were analyzed by centrifugation. Plasma corticosterone concentrations were measured by radio-immune-analysis (RIA kit of ICN Biomedical Inc.). Variation coefficients of the analysis, inter and intra assay, were 7 and 8 % respectively. Serum concentration of glucose and CK levels were analyzed by a Technicon multianalyzer (multichannel Technicon Analyzer RA-500), using commercial kits from Bayer Diagnostics, S. A. Plasmatic lactate concentration was measured using a Sigma Diagnostic Kit, by spectrophotometric process.

Carcasses were weighed while warm and at 24 h post mortem (at 1-2ºC). The mean weights of hot and cold carcasses in both treatments were 1192.50±121 g and 1175.26±120 g, respectively. To measure the pH24 of the Longissimus dorsi muscle, a portable pH meter (fitted with a Crison 52-00 penetration electrode) was used by making a small incision in the right loin. The pH metre was re-calibrated after every five recordings.

The descriptive statistics of the samples were analyzed using the technique of least squares, applied with a factorial model (GLM) including three fixed effects: transport time (1hT or 7hT), season (HT or CT) and position in the MFRS (top, middle or bottom). The cold weight of the carcass was introduced as a coverable and, in the absence of significance, was eliminated from the model. SAS statistical software (SAS, 1985) was used to perform the analyses.

RESULTS AND DISCUSSION

The average temperature during the journeys in summer was 27.16ºC (±1.93) with a relative humidity of 47.45% (±2.67). Average temperature in winter was 11.81ºC (±4.16) with relative humidity at 63.44% (±8.53). Changes in environmental conditions (temperatures and humidity) during the journeys are shown in Figure 1. Both temperature and humidity were observed to be stable during the summer, while variations were observed in winter. In both seasons temperature was not considered stressful as it stayed within the thermo-neutral zone for rabbits (between 10-30ºC; Animal Welfare Institute, 2004). The circumstances of the study could therefore be considered ideal for the analysis of normal commercial transport conditions.

Physiological blood stress parameters are shown in Table 1. The significance of each fixed effect, with their interactions, are shown in Tables 2 and 3. The minimum square means of every variable are shown according to the three fixed effects analyzed (transport time and season in Table 2, and position on the MFRS in Table 3) compared with the basal values obtained. All variables, except for hematocrite, were significantly affected by the treatments (as compared to basal values). Lactate and CK were twice as

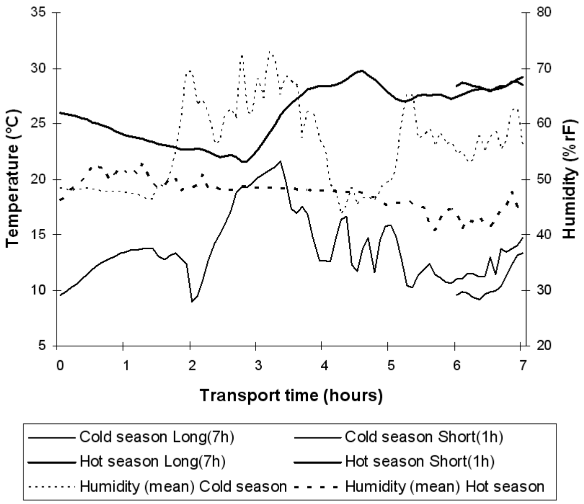

Figure 1: Graph of temperature and relative humidity according to transport time (7hT or 1hT) and season (HT or CT).

high as basal values, and corticosterone five times higher (>80ng/mL). Corticosterone values indicate a physiological stress response and a possible adaptive activity of animals during transport. Canali *et al.* (2000) also reported basal values of corticosterone of 20-27 ng/mL, as found in this study.

Transport time had a significant effect on corticosterone and CK values. These are two of the main parameters indicating physiological stress and even muscular damage in animals. Corticosterone and CK were higher in the 1hT than in 7hT, probably due to the animals' inability to adapt their homeostasis to the stress in a short time period. Canali *et al.* (2000) found higher cortisol values in animals transported for 6 h (>100ng/mL), compared to a control group. Glucose and CK levels were similar to other findings on transport stress (Canali *et al.*, 2000).

Season had a significant effect on corticosterone and CK values. Corticosterone was higher in the CT group (P<0.05), which disagrees with De la Fuente *et al.* (2004), who reported higher cortisol levels in animals transported in summer (20-30 ng/mL). Regarding stress from heat or noise, Verde *et al.* (1987) found higher corticosterone values than in our study (108.8 ng/mL for heat and 134.4 ng/mL for noise),

Table 1: Significance of the factorial model including fix effects of transport time (1hT or 7hT), season (HT or CT) and position on the MFRS (top, middle or bottom), on physiological stress indicators and pH24.

	Main effects[1]			Interactions			
	T	S	P	T×P	T×S	P×S	T×P×S
Hematocrite	NS	*	NS	NS	NS	NS	NS
Glucose	NS	NS	**	NS	NS	*	NS
Lactate	NS	NS	NS	NS	NS	*	NS
Corticosterone	*	*	**	**	**	NS	NS
CK	**	***	**	NS	NS	*	NS
pH24	NS	***	NS	NS	NS	NS	NS

[1]T, Transport time; S, Season; P, Position on the truck. * P<0.05; ** P<0.01; *** P<0.001;

Table 2: Least square means (±SE) of the physiological parameters analyzed according to the main effects of Transport time (7hT or 1hT) and Season (HT or CT).

		Transport time		Season	
	Basal	7 h (7hT)	1 h (1hT)	Summer (HT)	Winter (CT)
Hematocrite (%)	38.29±0.47	37.57±0.39^{a}	37.96±0.39^{a}	37.21±0.38^{a}	38.32±0.41^{b}
Glucose (mg/dL)	170±2.8 ^{x}	144.13±2.01^{ay}	147.45±1.98^{ay}	147.28±1.93^{ay}	144.3±2.05^{ay}
Lactate (mg/dL)	36.37±2.4 ^{x}	72.64±2.53$^{a\ y}$	75.02±2.49$^{a\ y}$	75.35±2.44^{ay}	72.31±2.59^{ay}
Corticosterone (ng/mL)	16.1±1.81 ^{x}	70.46±4.7^{ay}	84.48±4.64$^{b\ z}$	69.09±4.52$^{a\ y}$	85.85±4.82^{bz}
CK (UI/L)	1343±100 ^{x}	2631.9±107.8^{ay}	3041.4±106.3^{bz}	3522.3±103.7^{az}	2151.1±110.4^{by}
pH24	-	5.86±0.02^{a}	5.83±0.02^{a}	5.76±0.02^{a}	5.93±0.02^{b}

^{ab}Different lower-case letters in the same row indicate significant differences (*P*<0.05) between treatments. ^{xyz}Different superscripts in the same row indicate significant differences (*P*<0.05) between basal values and analyzed treatments.

but their treatments were more extreme. Animals submitted to HT treatment had significantly higher CK values (*P*<0.001). These results could be due to the thermal stress suffered by rabbits and to the sprinkling with water, which involved noise and rough handling of animals. Our results agree with De la Fuente *et al*. (2004), although they found lower levels (600-800 U/L). Glucose and lactate levels were slightly lower than those in the literature for high thermal stress (Verde *et al*., 1987; Abdelatif *et al*., 1994). However, Abdelatif *et al.* (1994) obtained CK values below 1000 U/L, while this study recorded values higher than 3000 U/L.

In regard to the position on the MFRS during transport, rabbits on the bottom position showed higher values of corticosterone than those in the middle (*P*<0.05), and the latter had higher levels than animals on the top (*P*<0.01). Glucose values were higher in the bottom and middle positions (*P*<0.01). No difference in lactate was found between levels. Rabbits allocated to the bottom and middle positions had significantly higher levels of CK (*P*<0.001), which, combined with the high lactate levels, demonstrates higher muscular activity. This may be due to the absence of visibility and the accumulation of dirt in these positions.

Carcass pH is used as a measure of meat quality and as an indicator of welfare. The pH at 24 hours post-mortem (pH24) was within the appropriate range for rabbits (5.50-5.80). Transport time and position did not affect pH24. These results agree with Trocino *et al*. (2002) and Xiccato *et al*. (1994). However,

Table 3: Least square means (±SE) of the physiological parameters analyzed according to the main effect of position on the truck (MFRS-top, middle or bottom).

		Position on the truck		
	Basal	Top	Middle	Bottom
Hematocrite (%)	38.29±0.47	37.70±0.50^{a}	37.84±0.49^{a}	37.74±0.44^{a}
Glucose (mg/dL)	170±2.8 ^{x}	139.47±2.55^{ay}	148.11±2.52^{by}	149.8±2.24^{by}
Lactate (mg/dL)	36.37±2.4 ^{x}	73.42±3.22^{ay}	70.9±3.18^{ay}	77.16±2.83^{ay}
Corticosterone (ng/mL)	16.1±1.81 ^{x}	64.82±5.98^{az}	79.55±5.91^{bz}	88.04±5.24^{cz}
CK (UI/L)	1343±100 ^{x}	2583.6±137.5^{ay}	3154.3±135.4^{bz}	2772.1±120.2^{cz}
pH24	-	5.83±0.02^{a}	5.86±0.02^{a}	5.85±0.02^{a}

^{abc}Different lower-case letters in the same row indicate significant differences (*P*<0.05) between treatments. ^{xyz}Different superscripts in the same row indicate significant differences (*P*<0.05) between basal values and analyzed treatments.

Table 4: Corticosterone interaction: Season×Transport. Least square means (±SE).

	Summer (HT)		Winter (CT)	
	1 h (1hT)	7 h (7hT)	1 h (1hT)	7 h (7hT)
Corticosterone (mg/mL)	70.88±6.22[a]	67.94±6.22[a]	68.63±6.22[a]	100.37±6.22[b]

[abc]Different lower-case letters in the same row indicate significant differences (P<0.05) between treatments.

other authors report higher pH24 (>6) in animals submitted to long periods of transport (Jolley, 1990; Masoero *et al*., 1992; Dal Bosco *et al*., 1997). Conversely, season did have a significant effect on pH24 (P<0.001). Animals transported in CT had pH24 values close to 6 (but not dark, firm and dry DFD meats) which indicates the onset of stress. These data coincide with De la Fuente *et al*. (2007) who found higher pH24 levels in animals transported in winter. Results show that extremely cold temperatures with highly fluctuating humidity had more of an effect on animal welfare than transport time.

Tables 4 and 5 show the interactions of corticosterone values between transport time and position on the MFRS, and between transport time and season. Cold temperatures caused more stress to animals during the 7hT transport because the rabbits were exposed to the stressor for a longer period. On the other hand, animals could better adapt to the conditions in the 1hT transport if placed on the top level of the MFRS because of better ventilation, more visibility and less dirt. Bottom or middle positions showed increased stress levels. Other interactions tested, between season and position on the MFRS for the parameters glucose, lactate and CK, do not seem to have a clear explanation.

Transport from farm to abattoir under extreme temperatures seems to be a significant stressor. The 1hT transport may not allow enough time for adaptation to the new situation. Transport time did not affect the pH24 but the cold season treatment had a high significant effect on pH24. The results indicate that season had a greater effect on physiological stress response than the transport time itself. This indicates the necessity for environmental control systems during transportation even if temperatures are inside the optimum values. In addition, position in the MFRS is an important factor to control since it seems to affect the welfare of the animals in the lower positions.

Our findings suggest that the transport process is a serious source of stress for the animals even under optimal conditions, as in this study. However, the magnitude of the stress did not overload the rabbits' adaptation system. t is important to invest in improvements related to the stress of animals during transport. Those improvements should be regulated through appropriate legislation that, according to the consumers' opinion, helps preserve the animals' welfare. It is also important to convince consumers that the ethical value of animal products is a rising idea in Europe and that the assurance of animal welfare will improve the image of the animal farm industry.

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Table 5: Corticosterone interaction: Transport time×Position on the truck. Least square means (±SE).

	7 h (7hT)			1 h (1hT)		
	Top	Middle	Bottom	Top	Middle	Bottom
Corticosterone (mg/mL)	71.75±8.46[a]	67.44±8.46[a]	72.20±7.49[a]	57.88±8.46[a]	91.67±8.25[b]	103.88±7.34[b]

[ab]Different lower-case letters in the same row indicate significant differences (P<0.05) between treatments.

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