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Physiological response of rabbits to heat, cold, noise and mixing in the context of transport

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Abstract

The effects on rabbits of four potential transport-related stressors (heat [HS], cold [CS], noise [NS] and mixing with unfamiliar animals [MS]) on certain physiological and meat quality parameters were studied. These are factors which may act to reduce the welfare of rabbits during their transport to the slaughterhouse. The rabbits were exposed to each potential stressor for four and a half hours prior to slaughter. HS groups showed the highest plasma concentrations of cortisol, lactate and glucose and greater packed cell volume (PCV) and osmolarity than the control group, and the meat exhibited a low initial pH as a direct consequence of lactic acid accumulation. The rabbits exposed to cold (CS) and noise (NS) showed physiological responses to the potential stressor, although to a lesser degree than rabbits exposed to heat. Cold stressed rabbits showed increased levels of creatine kinase (CK) and a higher PCV as well as decreased muscle glycogen concentration compared to the control. Rabbits exposed to noise showed muscular damage as demonstrated by increased levels of CK and lactate dehydrogenase (LDH) activity in the blood and a high final pH in meat. Mixing unfamiliar rabbits (MS) lead to higher CK activity, lower lactate and glucose concentration and the meat pH was slightly higher than the control group. In conclusion, these results suggested that rabbits exposed to heat were the most affected out of all three groups, although cold, noise and mixing with unfamiliar rabbits also had a detrimental effect on physiological and meat quality parameters.

Keywords: *animal welfare, cold stress, heat stress, mixing stress, noise stress, rabbit*

Introduction

Modern animal husbandry exposes rabbits (*Oryctolagus cuniculus*) to a variety of different stressors during their lives but transport is generally considered to be a particularly acute stressor, to which animals are subjected at least once during their life (Stephens 1982). Transport involves several potentially stressful factors: climatic factors such as temperature or humidity; physical factors, such as noise and vibration; and emotional factors, such as unfamiliar environment or social regrouping (Agnes *et al* 1990). All these factors can result in stress that is detrimental both to animal welfare and meat quality (in pigs: Lambooy *et al* 1987; Geverink *et al* 1998; calves: Agnes *et al* 1990; lambs: Ruiz de la Torre & Manteca 1999; Ibañez *et al* 2002; rabbits: Jolley 1990; Leoni *et al* 2000). Temperature as a stressor can negatively affect rabbits' productivity parameters (Crimella *et al* 1994; Marai *et al* 2002). Spain is subject to massive fluctuations in temperature throughout the seasons; thus, in summer, the temperature can be higher than 40ºC with a humidity lower than 40%, while in winter the temperature may not reach 5ºC and the humidity is about 60% (De la Fuente *et al* 2004). However, there is limited information about the effect of short-term heat and cold stress on the physiology of rabbits, in the context of transport.

It has been shown that noise may affect the behaviour and physiology of calves (Agnes *et al* 1990) and pigs (Forsling *et al* 1984); nonetheless, there is no scientific information about the effects of noise on rabbit welfare. It is known, however, that rabbits prefer low levels of sound, even though they have a degree of adaptation to high noise levels (Roca 1988). Rabbits rely strongly on odour for territorial marking (Mykytowycz 1968; González-Mariscal *et al* 1992), determination and reinforcement of dominance hierarchies and group membership (Mykytowycz *et al* 1976). Therefore, mixing unknown rabbits could be a stressful experience for them as unfamiliar odours could promote aggression (Jolley 1990).

Rabbit welfare during transport has not been as widely studied as in other domestic animals, and scientific papers about the effects of isolated factors as potential stressors on the physiology of rabbits are limited. The aim of the current experiment was to study the effects on welfare of four potential stressors typically linked with transport to the abattoir. Hence, four factors: heat, cold, noise, and mixing unfamiliar rabbits were applied separately and physiological and meat quality parameters were analysed.

Materials and methods

Ten trials were conducted to evaluate the effects of four potential stressors (heat, cold, noise and animal mixing) on

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rabbits' physiological and meat quality parameters. All the experimental procedures were approved by the Animal Experimentation Ethics Committee of the Veterinary Faculty of the Complutense University of Madrid, Spain.

Experimental animals

One hundred and twenty healthy Spanish Giant rabbits (an indigenous breed of Spanish rabbit) of both sexes (60 males and 60 females) with a mean live weight of 1.79 ± 0.02 kg, aged 55 - 65 days, were used throughout. The rabbits came from the experimental farm in the Veterinary Faculty of the Complutense University of Madrid. After weaning (aged 30 - 32 days), groups of six male and six female rabbits, each from a different litter, were housed twelve per cage in hanging wire cages (500 cm^2 per rabbit). A vitamin supplement was added to the rabbits' water one week after weaning. The food during the whole fattening period was offered *ad libitum* with a standard pelleted diet (16.0% protein, 2.5% fat, 16% fibre) and an automatic watering system was provided. The temperature inside the housing building was between 17 and 23ºC, the relative humidity was between 55 and 60%, the mean noise level was 72 ± 5 dB and light was provided by fluorescent tubes with a light programme of 16 h light and 8 h dark.

Experimental procedure

Ten trials, two for each potential stressor and the control were carried out. The trials for each potential stressor were performed on consecutive days, and control trials were performed at the end. Each fattening cage (6 males and 6 females) was assigned to each trial at random, in order to maintain the same fattening group in the experiment. The day before the commencement of the trial, the rabbits were gently removed from their own cages and transported in a box in groups of three to the experimental cage in the trial room; always by the same handler. The rabbits in each trial were exposed to the stressor for 4.5 h from 0800 to 1230h. During the trials, the rabbits were housed in an experimental cage made of steel wire 75 × 55 × 20 cm (length × breadth × height) (6,875 cm^3 per rabbit) and received neither food nor water. All trials were conducted in the same experimental room, which was 100 m from the rabbit house. This room was a cold storage facility that only worked in the cold stress trial. A heating system was provided for the rest of the trials to maintain the temperature within the range specified in each case. Adequate light and ventilation were provided throughout the experiment. The average temperature during the trials was 20 ± 3ºC and the relative humidity ranged from 50 to 55%, except in heat and cold stress trials. The mean noise level was 64 ± 5 dB, except in noise stress trials. The rabbits were weighed before and after each trial using an electronic digital balance (accuracy of ± 0.1 g).

The groups of rabbits exposed to each stress situation and the control groups were treated as follows:

Heat and cold stress (HS and CS) groups

Heat and cold were induced in the experimental room with controlled air temperature (accuracy of ± 2.0ºC). The inside temperature was 42.0 ± 2.0ºC in the heat experiments and –5.0 ± 2.0ºC in the cold experiments prior to the commencement of trials. The temperature sensors were placed inside the experimental cage. The sensors were air-range thermistor sensors of the type 'thermistor bead' (PCE Group Iberica, SL Tobarra, Spain), which had a stainless steel spun tip with a width of 3 mm and a length of 300 mm (accuracy of ± 0.1ºC). The sensor was connected to a data recorder which recorded the maximum and minimum temperatures during each trial. The maximum and minimum temperatures during the first heat trial were 44.8 and 35.2ºC respectively, and in the second trial 44.1 and 38.3ºC respectively. In the cold experiments, the maximum and minimum temperatures recorded were 2.1 and –1.1ºC respectively for the first trial, and 2.1 and –0.5ºC respectively for the second. The humidity ranged between 40 and 45% in the heat experiments, and between 55 and 60% in the cold experiments.

Noise stress (NS) group

The sound stressor was transmitted into the experimental room via two custom-built 220 watt speaker units (Arowana, PoSo International, UK). The experimental noise was recorded from a loaded commercial rabbit truck, then reproduced and amplified to achieve a sound pressure level of 96 dB.

Mixing stress (MS) group

Six rabbits (3 males and 3 females) from one cage, and six rabbits (3 males and 3 females) from another cage were put in the experimental cage at the start of each experiment. The two original cages had been separated from one another, in order to ensure that the animals from each cage were not familiar with one another.

Control group

The control rabbits were not exposed to any of the potential stressors or fasting. Twelve rabbits from two fattening cages, six from each (3 males and 3 females), were used. They were gently removed from the fattening cages and transported directly to the experimental abattoir immediately prior to slaughter.

At the end of each trial (4.5 h), 3 males and 3 females (6 rabbits × 2 trials = 12 rabbits for each analysed factor), chosen at random, were removed from experimental cages, stunned with low voltage (90 V for 2 s), and slaughtered in an experimental abattoir adjacent to the trial room. Blood samples were taken at exsanguination and drawn into EDTA and Sodium Fluoride (FNa) tubes. The EDTA tubes were used for analysing cortisol, creatine kinase (CK), lactate dehydrogenase (LDH), packed cell volume (PCV) and osmolarity, and the FNa tubes were used for analysing lactate and glucose. The blood samples in the FNa tubes were centrifuged at 3,000 rpm for 10 min within an hour of sampling. The tubes were refrigerated for analysis over the following 24 h.

The liver was weighed within 30 min of slaughter and a segment of the anterior lobe was frozen in liquid nitrogen for measurement of liver glycogen. A muscle sample of *m.*

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longissimus dorsi from between the 4th and 7th lumbar vertebra was taken at 30 min post mortem, on the right side and another one 24 h post mortem on the left to measure muscle glycogen concentration. Both muscle samples were frozen in liquid nitrogen for subsequent analysis.

Immediately post-slaughter, pH (pH_0) was measured on the left *m. longissimus dorsi* and *m. semitendinosus* muscles using a portable pH/temperature meter (Hanna Instruments HI-9025, Valencia, Spain). The carcase was then chilled to 4°C and 24 h after slaughter the pH (pH_{24}) was measured in these muscles from the right side.

Laboratory analyses

The blood samples collected into EDTA tubes were used within 24 h to analyse PCV. These blood samples were centrifuged at 6000 rpm for 5 minutes, and the plasma was separated and stored at 2ºC for subsequent analysis. Cortisol was measured by enzyme immunoassay (Radim, Roma). This method is 100% specific to cortisol and showed 1.2% cross reactivity with cortisone and 1.1% with corticosterone. Sensitivity was calculated based upon the standard curve, and taken as the minimal dose showing a significant difference from the mean plus two standard deviations. The dose was 5.0 ng ml^{-1}. The inter-assay precision was calculated from replicate analyses (n = 10) of quality controls. The inter-assay coefficients of variations for plasma quality controls at concentrations 254.2, 156.6 and 29.2 ng ml^{-1} were 6.1, 4.3 and 6.9%, respectively. The intra-assay precision was < 7% for cortisol concentrations between 0.5 and 150 ng ml^{-1}. Levels of CK and LDH were measured on an SBA autoanalyser, using Boehringer Mannhein reagents. The ion lactate and glucose concentrations were measured by enzymatic-spectrophotometric methods (Spinreact, SA, Sant Esteve de Bas, Spain). The PCV was assessed by the microhaematocrit technique. The osmolarity was calculated using the following equation (Balcells 1995):

$$(1.86 \times \text{Sodium}) + \frac{(\text{Glucose})}{18} + \frac{(\text{Urea})}{38.3} + 9$$

The sodium and urea concentrations were measured by flame photometric and enzymatic-spectrophotometric methods, respectively. The glycogen concentrations in liver and muscle were assessed according to the technique described by Dreiling *et al* (1987).

Statistical analyses

All analyses were performed using Statgraphics Plus (1994) software. The five groups, HS, CS, NS, MS and control, were compared using one-way analysis of variance, and the Student-Newman-Keuls method was used for multiple means comparison. Each rabbit was considered as one experimental unit. Gender (male and female) was not included in the model since in pre-pubertal rabbits the difference between genders is minimal (Jolley 1990). The two trials from each group were first compared using an unpaired *t*-test to determine whether or not they were significantly different. In order to assess the variables' normality, Shapiro-Wilk test for normality of residuals, and Cochran's *C*-test for homogeneity of variances were performed. Plasma cortisol, lactate and glucose concentrations, and plasma CK activity were normally distributed when $\log_{10}$ transformed. The other parameters were normally distributed without transformation.

Table 1 Effect of stressor on live weight loss (mean ± SEM).

	Potential stressor			
	Heat (n = 12)	**Cold (n = 12)**	**Noise (n = 12)**	**Mixing (n = 12)**
Live weight losses (%)	3.03 ± 0.19	2.61 ± 0.18	2.67 ± 0.55	3.54 ± 0.25

Results

There were no significant ($P > 0.05$) differences in live weight losses during the trials between the four groups exposed to potential stressors. The control group was not included in this statistical analysis because they did not fast (Table 1).

The highest plasma cortisol concentration was found in the HS group. NS rabbits also showed higher cortisol concentration than CS group. However, the MS and control group did not differ significantly in terms of their cortisol concentration (Table 2). The plasma CK concentration was higher ($P < 0.05$) in each of the groups exposed to stressors, compared to the control group, with activity highest in the MS group and significantly different to NS, HS and CS groups. The lowest CK activity was in the CS group (Table 2).

Only the NS group showed increased levels of LDH with no difference being seen between the control and the HS, CS and MS groups. The greatest plasma concentrations of lactate and glucose were seen in rabbits exposed to heat (HS). The lowest concentration of plasma lactate was for CS and control groups, whereas MS showed the lowest plasma glucose concentration (Table 2). The HS, NS and CS groups had the highest PCV and the values differed statistically from the MS and control groups. HS followed by NS provided the highest osmolarity. There were no statistically significant differences in PCV and osmolarity between the control group and MS (Table 2).

In Table 3 mean liver weight and liver and muscle glycogen levels are presented. Liver weight was significantly higher in the HS group compared to the others, while the lowest levels of liver glycogen were found in HS, NS and CS rabbits. Muscle glycogen concentration at 30 min was lower in the CS group than the other four and this trend was also seen at 24 h post mortem, although no longer at a statistically significant level compared to HS and NS groups. MS and control groups showed the highest muscle glycogen concentration after chilling.

The means of the pH_0 and pH_{24} and the differences between them are presented in Table 4. HS had the lowest pH_0 values

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Table 2 Effect of stressor on blood parameters (mean ± SE).

	Potential stressor				Control (n = 12)	*P* value
	Heat (n = 12)	Cold (n = 12)	Noise(n = 12)	Mixing (n = 12)		
Cortisol (log_{10} µg dl^{-1})	1.46^{c} ± 0.07	0.92^{a} ± 0.04	1.19^{b} ± 0.05	0.95^{ab} ± 0.11	0.97^{ab} ± 0.05	< 0.0001
CK (log_{10} IU l^{-1})	3.55^{c} ± 0.04	3.42^{b} ± 0.02	3.59^{c} ± 0.04	3.72^{d} ± 0.05	3.26^{a} ± 0.02	< 0.0001
LDH (IU l^{-1})	794.5^{a} ± 105.7	627.3^{a} ± 30.3	$1,308.7^{b}$ ± 92.7	501.0^{a} ± 82.2	694.9^{a} ± 44.1	< 0.0001
Lactate (log_{10} m mol l^{-1})	1.89^{c} ± 0.03	1.56^{a} ± 0.02	1.65^{b} ± 0.02	1.64^{b} ± 0.03	1.53^{a} ± 0.02	< 0.0001
Glucose (log_{10} mg dl^{-1})	2.38^{c} ± 0.04	2.23^{b} ± 0.02	2.34^{c} ± 0.01	1.82^{a} ± 0.10	2.22^{b} ± 0.03	< 0.0001
PCV (%)	42.3^{c} ± 0.9	37.9^{b} ± 0.9	41.5^{c} ± 0.9	33.6^{a} ± 0.8	34.9^{a} ± 0.8	< 0.0001
Osmolarity (m osm l^{-1})	305.8^{c} ± 5.4	267.0^{a} ± 2.3	289.9^{b} ± 1.6	260.5^{a} ± 3.1	265.1^{a} ± 3.0	< 0.0001

Significant differences: ^{abc} $P < 0.05$.

in *m. longissimus dorsi* and *m. semitendinosus*, though the latter did not differ significantly from the control. After chilling, the control rabbits had the lowest pH_{24} in both muscles; whereas the NS group had the highest value. With regard to differences between pH_0 and pH_{24}, MS and control groups had a higher fall than the other three groups (HS, CS and NS) in both muscles.

Discussion

One of the main factors leading to weight loss during transport is fasting, although initial losses are brought about through a combination of defaecation and urination (Knowles *et al* 1995). In rabbits, Coppings *et al* (1989) reported that food or water deprivation for 12 or 24 h had no adverse effects on weight, whereas significant weight losses were seen after 36 h. In the present study, all groups (except the control) fasted for 4.5 h, and no significant differences in live weight losses were seen among all four treatments. Ashby *et al* (1980) used an equation to evaluate live weight loss of rabbits during fasting. When that equation was applied to this study, the live weight loss was underestimated (1.4%) as reported by Jolley (1990) and De la Fuente *et al* (2004). This could be because the rabbits were simultaneously exposed not only to fasting, but to another potential stressor. De la Fuente *et al* (2004) and Purdue (1984) found that rabbits exposed to both fasting and transport showed more live weight loss than those that merely fasted. Masoero *et al* (1992) reported a live weight loss of 2.2% for rabbits transported for 2 h. This was lower than our findings, perhaps as a result of the timeframe of our trial (4.5 h).

Temperature could have an impact on live weight loss, but we found no significant differences between rabbits subjected to either heat or cold and those not subject to temperature change. However, Luzi *et al* (1994) found less weight loss in rabbits transported at 0 and 6ºC than at 15ºC (1.5 as opposed to 3.9%). Abdelatif and Modawi (1994) found bodyweight losses of 3.0 and 3.3% in rabbits exposed to 45 and 50ºC respectively over 6 h. It has also been reported that time of year can significantly affect live weight loss during transport (De la Fuente *et al* 2004). The live weight losses observed here lie within the range reported by these authors.

The temperatures during the heat trial may have been higher than rabbits' upper critical temperatures but they were at a level frequently seen during the summer months in many Mediterranean countries (De la Fuente *et al* 2004). Nevertheless, they did not generate sufficient heat stress to produce heat stroke in the rabbits, since no rabbit died. Temperatures over 42ºC have been known to produce heat stress in rabbits, increasing cortisol concentration (Abdelatif & Modawi 1994). The highest plasma cortisol concentration for the HS group is in accordance with previous findings (De la Fuente *et al* 2004), who reported higher cortisol levels in rabbits transported in summer than in winter.

CK and LDH are two enzymes associated with the stress response and physical fatigue (Perez *et al* 2002; Broom *et al* 1996). Only the noise group showed significantly higher levels of LDH. An increase in plasma CK seems to be associated with alterations in the permeability of the cell membrane, brought about by changes to tissue temperature (Manjoo *et al* 1985). The CK concentration was significantly higher for rabbits exposed to the four potential stressors than the control group. Noise can lead to muscle damage due to enhanced ambulatory activity, as in pigs (Talling *et al* 1996), which showed a greater than fourfold ambulation score compared to control pigs after exposure to a noise level of 97 dB. A similar process could explain the high CK levels in mixed rabbits. In addition, Abdelatif and Modawi (1994) observed that heat increased CK activity threefold when the rabbits' rectal temperature was increased from the normal value of 39.4 to 43.1ºC. In CS rabbits the increase in CK may have been related to increased muscular activity for enhancing heat production and raising body temperature. The high plasma lactate concentration in HS rabbits could be attributed to an accumulation of lactic acid due to excessive panting. Rabbits have a limited capacity for losing heat by sweating and panting (evaporative heat loss); moreover panting remains an inefficient mode of heat loss when the environmental temperature is in excess of 30ºC (Fayez *et al* 1994). If panting is prolonged, a metabolic alkalosis can occur due to CO_2 deficit. In certain instances, when thermal polypnoea is insufficient to reduce body temperature, the animal can go into circulatory shock,

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Table 3 Effect of stressor on liver weight and muscle and liver glycogen concentration (mean ± SE).

	Potential stressor				Control (n = 12)	*P* value
	Heat (n = 12)	Cold (n = 12)	Noise (n = 12)	Mixing (n = 12)		
Liver weight (g)	67.4^{b} ± 5.1	47.0^{a} ± 1.8	48.0^{a} ± 1.2	54.7^{a} ± 1.4	56.1^{a} ± 1.5	< 0.0001
Liver glycogen (mg g^{-1} tissue)	93.8^{a} ± 27.7	53.7^{a} ± 33.3	53.3^{a} ± 20.1	330.7^{b} ± 14.5	295.8^{b} ± 30.6	< 0.0001
Muscle glycogen (mg g^{-1} tissue)						
30 min[l]	7.34^{b} ± 0.49	5.07^{a} ± 0.53	7.27^{b} ± 0.71	8.92^{b} ± 0.40	7.63^{b} ± 0.39	0.0001
24 h[l]	0.67^{ab} ± 0.10	0.41^{a} ± 0.10	0.70^{ab} ± 0.17	1.08^{b} ± 0.18	1.12^{b} ± 0.24	< 0.0154
Difference	6.67^{b} ± 0.41	4.65^{a} ± 0.48	6.57^{b} ± 0.57	7.84^{b} ± 0.26	6.50^{b} ± 0.29	0.0001

Significant differences: ^{ab} $P < 0.05$.
[l] Time after slaughter that measurements were made.

Table 4 Effect of stressor on pH of *longissimus dorsi* and *semitendinosus* muscles (mean ± SE).

	Potential stressor				Control (n = 12)	*P* value
	Heat (n = 12)	Cold (n = 12)	Noise (n = 12)	Mixing (n = 12)		
Longissimus dorsi						
pH_0	6.39^{a} ± 0.06	6.77^{b} ± 0.04	6.80^{b} ± 0.05	6.79^{b} ± 0.06	6.60^{b} ± 0.03	< 0.0001
pH_{24}	5.76^{b} ± 0.03	5.94^{c} ± 0.02	6.07^{d} ± 0.02	5.83^{b} ± 0.03	5.67^{a} ± 0.03	< 0.0001
Difference	0.63^{a} ± 0.06	0.82^{ab} ± 0.05	0.72^{a} ± 0.06	0.96^{b} ± 0.07	0.93^{b} ± 0.04	0.0035
Semitendinosus						
pH_0	6.25^{a} ± 0.13	6.66^{b} ± 0.03	6.68^{b} ± 0.05	6.74^{b} ± 0.05	6.34^{a} ± 0.02	< 0.0001
pH_{24}	6.13^{c} ± 0.03	6.12^{c} ± 0.03	6.34^{d} ± 0.05	5.97^{b} ± 0.04	5.81^{a} ± 0.01	< 0.0001
Difference	0.12^{a} ± 0.10	0.54^{bc} ± 0.03	0.40^{b} ± 0.06	0.71^{c} ± 0.07	0.54^{bc} ± 0.02	< 0.0001

Significant differences: ^{abcd} $P < 0.05$.

leading to hypotension and metabolic acidosis; thereby causing a build-up of lactic acid in the bloodstream (Castaño-Bello 1995). NS and MS groups both showed greater lactate concentrations that may have been the result of enhanced muscular activity brought about by increased ambulation in these rabbits.

The higher plasma glucose concentrations found in HS rabbits are in accordance with the findings of Abdelatif and Modawi (1994) who reported hyperglycaemia in rabbits exposed to heat stress. This could, perhaps, be attributed to an inhibition of insulin secretion under heat stress. Moreover, these rabbits had lower levels of liver glycogen as a direct result of heat-induced hepatic glycogenolysis.

High temperatures can induce haemoconcentration, which is associated with the increase in evaporative water loss (Abdelatif & Modawi 1994). Also, stress can lead to the release of blood cells into the circulation as a result of sympatho-adrenal stimulation leading to contraction of the spleen (Crookshank *et al* 1979; Perez *et al* 2002). HS, NS and CS rabbits had a higher PCV and osmolarity than the control group, and osmolarity was higher in HS and NS. Therefore, these groups showed signs of dehydration. Previous papers have reported an increase in osmolarity when rabbits were transported in summer (De la Fuente *et al* 2004), whereas Abdelatif and Modawi (1994) did not find any effect on the PCV of rabbits exposed to heat stress.

The duration of fasting is apparently one of the main factors determining liver weight (Jolley 1990), although Purdue (1984) reported higher liver weights in transported rabbits than in fasted ones (after 24 h). Since the liver is involved in regulating blood glucose levels (Jolley 1990), the increased liver weight in HS rabbits may be explained by increased metabolic activity under heat stress. The low glycogen concentration in liver and muscle in CS rabbits could be due to increased activity associated with heat production, and is in accordance with other authors (De la Fuente *et al* 2004).

Pre-slaughter handling can influence the final pH (Hulot & Ouhayoun 1999), causing an increase and producing dark meat (Dalle Zotte & Ouhayoun 1995). In addition, HS had the lowest pH_0 due to metabolic acidosis, in which lactic acid is seen to accumulate in tissues, including muscle. There were no significant differences in pH_0 between CS, NS, MS and control groups, and values were similar to normal pH values previously reported for rabbits (Dal Bosco *et al* 1997; Paci *et al* 1999; Hulot & Ouhayoun 1999).

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The higher pH_{24} in the NS group could be a consequence of greater muscle activity in these rabbits, since they also had higher plasma LDH concentration. The control group had pH_{24} values, in both muscles, similar to those reported by other authors (Dalle Zotte & Ouhayoun 1995; Paci *et al* 1999; Piles *et al* 2000).

The four potential stressors had a graduated effect on rabbit welfare. Heat appeared to be the most stressful factor as it lead to the highest plasma concentrations of cortisol, as well as all other analysed parameters.

Exposure to noise produced muscular damage as demonstrated by an increase in plasma concentrations of CK and LDH, and the high final meat pH. Rabbits subjected to heat and noise also showed signs of dehydration.

HS, CS and NS rabbits showed depleted liver glycogen, but only cold reduced muscle glycogen. The meat quality of these rabbits was also affected, since the values of pH_0, pH_{24} and pH fall were affected.

The mixing of unfamiliar rabbits did not appear to significantly effect physiological responses; blood parameters and glycogen concentration remaining similar to the controls, although pH was higher. This is consistent with rabbits in meat production being transported to the abattoir while still pre-pubertal. Unfamiliar odours would be expected to have less of an effect on young rabbits compared to adults (Jolley 1990; González-Mariscal *et al* 1992).

The analysis of one isolated stressor does not provide complete information upon which to base comprehensive solutions to stress responses generated by transport. Studying a variety of stressors separately has limited application to the dynamic, potentially interacting complex of stressors that exist (Abeyesinghe *et al* 2001). A more comprehensive approach to studying potential stressors would be to systematically characterise the magnitude and frequency of each stressor event (Mitchell & Kettlewell 1998) and determine their separate and combined effects (Weeks & Nicol 2000). Therefore, more research is needed to study the impact of several transport-related factors, acting together, on animal welfare.

Animal welfare implications

Heat stress has a significant effect on rabbit welfare, while the effects of cold and noise are less marked, producing only superficial fatigue and muscular damage. Thus, temperature and ventilation should be controlled during rabbit transport in order to avoid heat stress as it induces a considerable stress response. Of the remaining potential stressors, cold and noise, should also be controlled though their potential effect is lower. Mixing rabbits of two months old does not appear to affect welfare as Jolley (1990) suggested.

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References

Abdelatif AM and Modawi SM 1994 Effects of hyperthermia on blood constituents in the domestic rabbits. *Journal of Thermal Biology 19:* 357-363

Abeyesinghe SM, Wathes CM, Nicol CJ and Randall JM 2001 The aversion of broiler chickens to concurrent vibrational and thermal stressors. *Applied Animal Behaviour Science 73:* 199-215

Agnes F, Sartorelli P, Hagi Abdi B and Locatelli A 1990 Effect of transport loading or noise on blood biochemical variables in calves. *American Journal of Veterinary Research 51:* 1679-1681

Ashby BH, Ota H, Bailey A, Whitehead JA and Kindya WG 1980 Heat and weight loss of rabbits during simulated air transport. *Transcription ASAE 23:* 162-164

Balcells A 1995 Examen de sangre; Química hemática. In: *Clínica y Laboratorio 15th edition* pp 57-143. Massan-Salvat: Barcelona, Spain. [Title translation: Blood examination; blood chemistry]

Broom DM, Gooder JA, Hall SJG, Lloyd DM and Parrott RF 1996 Hormonal and physiological effects of 15 hour journey in sheep: comparison with the responses to loading, handling and penning in the absence of transport. *British Veterinary Journal 152:* 593-604

Castaño-Bello H 1995 Control y regulación de la temperatura corporal. In: García Sacristan A (ed) *Fisiología Veterinaria* pp 1015-1025. McGraw-Hill: Madrid, Spain. [Title translation: Control and regulation of body temperature]

Coppings RJ, Ekhator N and Ghodrati A 1989 Effects of antemortem treatment and transport on slaughter characteristics of fryer rabbits. *Journal of Animal Science 67:* 872-880

Crimella C, Heinz E and Luzi F 1994 Performance of rabbits in hot climate: a housing "cooling system" for heat stress reduction. *Options Méditérrannéennes 8:* 463-467

Crookshank HR, Elissalde MH, Whie RG, Clanton D and Smalley HE 1979 Effect of transportation and handling of calves upon blood serum composition. *Journal of Animal Science 48:* 430-435

Dal Bosco A, Castellini C and Bernardini M 1997 Effect of transport and stunning method on some characteristics of rabbit carcasses and meat. *World Rabbit Science 5:* 115-119

Dalle Zotte A and Ouhayoun J 1995 Post-weaning evolution of muscle energy metabolism and related physico-chemical traits in the rabbit. *Meat Science 39:* 395-401

De la Fuente J, Salazar MI, Ibáñez M and González de Chavarri E 2004 Effects of season and stocking density during transport on live weight and biochemical measurements of stress, dehydration and injury of rabbits at time of slaughter. *Animal Science 78:* 285-292

Dreiling CE, Brown DE, Casale L and Kelly L 1987 Muscle glycogen: comparison of iodine binding and enzyme digestion assays and application to meat samples. *Meat Science 20:* 167-177

Fayez I, Marai M, Alnaimy A and Habeeb M 1994 Thermoregulation in rabbits. *Options Méditérrannéennes 8:* 33-41

Forsling ML, Sharman DF and Stephens DB 1984 Vasopressin in the blood plasma of pigs and calves exposed to noise and vibration comparable with that experienced during transport. *Journal of Physiology 357:* 96-103

Geverink NA, Bühnemann A, Van de Burgwal JA, Laambooy E, Blokhuis HJ and Wiegant VM 1998 Responses of slaughter pigs to transport and lairage sound. *Physiology & Behavior 63:* 667-673

González-Mariscal G, Melo AI, Zavala A and Beyer C 1992 Chin-marking behaviour in male and female New Zealand rabbits: Onset, development, and activation by steroids. *Physiology & Behavior 52:* 889-893

https://doi.org/10.1017/S0962728600030918 Published online by Cambridge University Press

Hulot F and Ouhayoun J 1999 Muscular pH and related traits in rabbits: a review. *World Rabbit Science 7:* 15-36

Ibañez M, De la Fuente J, Thos J and Gónzalez de Chavarri E 2002 Behavioural and physiological responses of suckling lambs to transport and lairage. *Animal Welfare 11:* 223-230

Jolley PD 1990 Rabbit transport and its effects on meat quality. *Applied Animal Behaviour Science 28:* 119-134

Knowles TG, Brown SN, Warriss PD, Phillips AJ, Dolan SK, Hunt P, Ford JE, Edwards JE and Watkins PE 1995 Effects on sheep of transport by road up to 24 hours. *Veterinary Record 136:* 421-438

Lambooy E, Van der Hel W, Hulsegge B and Brandsma HA 1987 Effect of environmental temperature and air velocity two days preslaughtering on heat production, weight loss and meat quality in non-fed pigs. In: Verstegen MWA and Henken AM (eds) *Energy Metabolism in Farm Animals: Effects of housing, stress and disease* pp 164-179. Martinus Nijhoff Publishers: Dordrecht, The Netherlands

Leoni S, Moriggi F and Ghilarducci G 2000 Transporto e qualità della carne. *Rivista di Coniglicoltura 3:* 40-47. [Title translation: Transport and meat quality]

Luzi F, Heinzl E, Crimella C and Verga M 1994 Influence des conditions de transport sur la qualité des carcasses. *Cuniculture 120:* 277-279. [Title translation: Influence of transport conditions on carcass quality]

Manjoo M, Burger FJ and Kielblock AJ 1985 A relationship between heat load and plasma enzyme concentration. *Journal of Thermal Biology 10:* 221-225

Marai IFM, Habeeb AAM and Gad AE 2002 Rabbits' productive, reproductive and physiological performance traits as affected by heat stress: a review. *Livestock Production Science 78:* 71-90

Masoero G, Riccioni L, Bergoglio G and Napolitano F 1992 Implications of fasting and of transportation for a high quality rabbit meat product. *Journal of Applied Rabbit Research 15:* 841-847

Mitchell MA and Kettlewell PJ 1998 Physiological stress and welfare of broiler chickens in transit: solutions not problems! *Poultry Science 77:* 1803-1814

Mykytowycz R 1968 Territorial marking by rabbits. *Scientific American 218:* 116-126

Mykytowycz R, Hersterman ER, Gambale S and Dudzinski ML 1976 A comparison of effectiveness of the odours of rabbits, *Oryctolagus cuniculus*, in enhancing territorial confidence. *Journal of Chemical Ecology 2:* 13-24

Paci G, Marzoni M, Piloni S and Bagliacca M 1999 Effetto della stagione e della tecnica de allevamento sulle prestazioni produttive e sulla qualità della carne di coniglio. *Revista di Coniglicoltura 9:* 30-36**.** [Title translation: Effect of season and stunning method on productive parameters and meat quality of rabbit]

Perez MP, Palacio J, Santolaria MP, Aceña MC, Chacón G, Verde MT, Calvo JH, Zaragoza MP, Gascón M and García-Belenguer S 2002 Influence of lairage time on some welfare and meat quality parameters in pigs. *Veterinary Research 33:* 239-250

Piles M, Blasco A and Pla M 2000 The effect of selection for growth rate on carcass composition and meat characteristics of rabbits. *Meat Science 54:* 347-355

Purdue D 1984 *The effect of transport and fasting on physiological indices of stress and subsequent meat quality of the rabbit.* MSc Thesis, University of Bristol, UK

Roca A 1988 Alojamientos e instalaciones en cunicultura. In: Sanz C, Buxadé I and Ovejero E (eds) *Bases para el diseño de alojamientos e instalaciones ganaderas* pp 173-193. Asociación de Ingenieros Agrónomos de Cataluña: Barcelona, Spain. [Title translation: Housing and equipment for rabbit]

Ruiz de la Torre JL and Manteca X 1999 Behavioural effects of social mixing at different stocking densities in prepubertal lambs. *Animal Welfare 8:* 117-126

Statgraphics Plus 1994 *Statgraphics Plus: Reference Manual, Version 4.* Statistical Graphics Corporation: Maryland, USA

Stephens DB 1982 Transport of animals intended for breeding, production and slaughter. In: Moss R (ed) *Transport of animals intended for breeding, production and slaughter* pp 187-206. Martinus Nijhoff Publishers: The Hague, The Netherlands

Talling JC, Waran NK, Wathes CM and Lines JA 1996 Behavioural and physiological responses of pigs to sound. *Applied Animal Behaviour Science 48:* 187-202

Weeks C and Nicol C 2000 Poultry handling and transport. In: Grandin T (ed) *Livestock, handling and transport 2nd edition* pp 363-384. CABI Publishing: Wallingford, UK

https://doi.org/10.1017/S0962728600030918 Published online by Cambridge University Press