Irish Journal of Agricultural and Food Research **45:** 39–50, 2006

Effects of transporting bulls at different space allowances on physiological, haematological and immunological responses to a 12-h journey by road

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The effects of space allowance during transportation on physiological, haematological and immunological responses in nine-month old bulls (250 kg) were assessed before and after a 12-h road journey. Following transport, animals transported at a spatial allowance of 1.27 m^2 had higher ($P \leq 0.001$) non-esterified fatty acid concentrations than control. The stimulated production of interferon-γ, in response to concanavalin-A and keyhole limpet haemocyanin, and plasma cortisol were not different at the 0.85 m^2 and 1.27 m^2 stocking densities. Glucose and albumin concentrations were higher ($P \leq 0.001$) post-transport in all transported animals than control. The percentage lymphocytes was reduced ($P \leq 0.001$) and neutrophil percentage and the number of neutrophils were increased ($P \leq 0.001$) in all transported treatments. There were no changes ($P > 0.05$) in monocyte numbers, monocyte percentage or platelet numbers following transportation. The haematocrit values were higher ($P \leq 0.001$) in the transported treatments while RBC numbers were higher ($P \leq 0.001$) in the animals transported at a spatial allowance of 1.27 m^2 than control. Protein, globulin, urea and lactate concentrations, and white blood cell numbers were not changed at any time during the study. The concentration of β-hydroxybutyrate was lower ($P \leq 0.001$) in all animals following transport. Plasma haptoglobin concentrations were unchanged following transportation while plasma fibrinogen concentrations were reduced in all transported treatments. There were no differences among treatments in rectal temperature or live weights pre- and post-transport. The results indicate that within the conditions of the study, there was no welfare advantage in transporting bulls at 1.27 m^2 versus the standard spatial allowance of 0.85 m^2 on a 12-h road journey.

Keywords: Animal welfare; haematology; immunology; physiology; transport

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Introduction

The effects of transport on the welfare of cattle and calves have been the subject of detailed studies to evaluate and identify factors affecting the welfare of animals during and after transport (Tarrant, 1990; Trunkfield and Broom, 1990; Knowles, 1995a,b; Knowles *et al*., 1993; 1994a; 1994b; Tarrant, Kelly and Harrington, 1988; Tarrant and Grandin, 2000; Tarrant, 1990; Grigor *et al*., 2001). The Scientific Committee on Animal Health and Welfare (SCAHAW, 2002), advising the European Commission, adopted a report on the welfare of animals during transport. SCAHAW (2002) advised on maximum travel and resting times, watering and feeding intervals, stocking densities and loading methods. The SCAHAW report (2002) showed that the scientific basis for several of the EU regulations (e.g., EC 91/628; EC 98/411) was weak and where there are data, there are different opinions regarding conclusions to be drawn. A clear disadvantage was that the recommendations were often based on the results of one treatment group of animals, which is unlikely to represent Europe as a whole, and it is clear that most of the work on transport has been carried out in Northern European countries, which does not include the extremes of climate possible within Europe. Furthermore, there was no scientific evidence on which to base guidelines for spatial allowance, as most are based on the animals' size and on practical experience. The Farm Animal Welfare Council (FAWC) (1991) produced the formula $A = 0.021\ W^{0.67}$ for calculating the minimal spatial area (m^2) for each animal based on live weight: where W = live weight (kg). Using published guidelines for spatial allowance from other sources, Randall (1993) derived the equation where $A = 0.01\ W^{0.78}$; however, Randall (1993) recommended the use of the equation given by FAWC because it was more generous in its space allowance for larger animals. The SCAHAW report (2002) recommended that "for journeys in which a period for rest, feeding, and drinking is needed, this rest should be on the vehicle so the formula $A = 0.0315\ W^{0.67}$ should be used". Studies on cattle housed at different space allowances reported either no changes in the basal cortisol concentrations (Fisher *et al*., 1997a), increased (Friend *et al*., 1977) or decreased (Benek *et al*., 1984; Fisher *et al*., 1997b) cortical responses to exogenously administered adrenocorticotrophic hormone (ACTH). Hickey, Earley and Fisher (2003) reported attenuation of lymphocyte proliferation in *ex vivo* immune functions tests for cattle at <2 m^2 space allowances. In contrast, Fisher *et al*. (1997a) found no differences between space allowances for 1.5, 2.0, 2.5 and 3 m^2 per heifer for *ex vivo* cellular immune function. However, there is limited or no published experimental work on which to base recommendations for spatial allowance for cattle during transport. In a series of studies by Kenny and Tarrant (1987a), the welfare of groups of Friesian steers (582 kg live weight) was evaluated when animals were subjected to either loading and unloading, confinement for 1 h on a stationary truck, or transport on the same truck for 1 h. Only heart rate increased in response to loading and unloading, whereas confinement on the stationary vehicle increased plasma cortisol, glucose and creatine kinase concentrations. In comparison with the stationary treatment, cattle that were transported had decreased social interactions, a higher rate of defaecation, and were less successful in changing position. In a similar study with bulls (Kenny and Tarrant, 1987b), social re-grouping and re-penning, as if in preparation for transportation, did not

have any consistent effects on plasma cortisol, while confinement on a stationary vehicle increased plasma cortisol concentrations and actual transportation resulted in still higher cortisol concentrations.

The overall objective of the present study was to investigate the physiological, haematological and immunological responses of nine-month old bulls (250 kg) to transport under current EU legislation (EU 91/628) (0.85 m^2) and the proposed spatial allowance 1.27 m^2 (SCAHAW, 2002) on a 12-h journey by road.

Materials and Methods

Twenty-nine bulls (mean live weight 250 (s.d. 53) kg) were randomly assigned to one of three treatments: Control (C) not subject to transport (n = 16); T085 – transported at 0.85 m^2 (n = 16); T127 – transported at 1.27 m^2 (n = 13). Transport involved a 12-h journey by road. Bulls assigned to the control treatment were housed on slats (2 m^2/animal) and offered silage *ad libitum* plus concentrates (2 kg/head) at Grange Research Centre. The bulls assigned to the transport treatments were loaded onto the lower deck of an articulated transporter (total area = 30.96 m^2) which was divided into 4 fan-ventilated pens, going in the direction from front to rear of the transporter, with spatial allowances per animal of 0.85 m^2 (pen 1), 1.27 m^2 (pen 2), 0.85 m^2 (pen 3) or 1.27 m^2 (pen 4).

On the evening of the journey (8 July, 2002), all animals were blood sampled (day 0; pre-transport) by jugular venipuncture to provide baseline physiological, haematological and immunological measurements. The bulls were weighed and randomly allocated, at 18:00, to pens on the transporter and transported on a 12-h journey. The individual pens on the transporter were bedded with sawdust, and water was available through nipple drinkers. The 12-h journey from Grange Research Centre to County Cork, and the return journey (608 km), involved a combination of road surfaces ranging from motorways, secondary roads to small country lanes. On completion of the journey, blood samples were collected by jugular venipuncture (day 1; post-transport) for physiological and haematological measurements. The control animals were also sampled at this stage.

Physiological, haematological and immunological variables

Heparinised blood samples were collected by jugular venipuncture and the plasma was separated by centrifugation at 1,600 $\times g$ (300 $\times g$ for interferon-γ) at 8 °C for 15 min, and subsequently stored at –20 °C until assayed for: cortisol, glucose, lactate, non-esterified fatty acids (NEFA), β-hydroxy butyrate (BHB), urea, total protein, albumin, creatine phosphokinase (CPK), lactate dehydrogenase (LDH), and the acute-phase proteins (fibrinogen and haptoglobin). Blood samples for interferon-γ (IFN-γ) determination were collected by jugular venipuncture into aseptic vacutainer tubes containing lithium heparin and the stimulated lymphocyte production of IFN-γ was determined following whole blood culture. The lymphocyte production of IFN-γ was determined (Earley and Crowe, 2002) following stimulation *in vitro* with either phosphate buffered saline alone, keyhole limpet hemocyanin (KLH; 20 μg/1.5 mL blood), or concanavalin A (Con-A; 20 μg/1.5 mL blood) in whole blood culture; the IFN-γ concentration in the harvested plasma samples was measured using a specific ELISA procedure (CSL, Biosciences, Parkville, Victoria, Australia).

The haematological variables (red blood cell number (RBC), haemoglobin (Hb), haematocrit (packed cell volume

(PCV)), mean cell volume (MCV), total white cell (WBC) count, percentages of granulocytes, monocytes and lymphocytes platelet number, mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) were determined on unclotted (K_3-EDTA) whole-blood samples using an electronic particle hematology analyser (Celltac MEK-610K, Nihon Kohden, Japan). Plasma cortisol concentration was determined using a commercially available radioimmunoassay kit. Plasma haptoglobin concentration was measured by determining the haemoglobin-binding capacity using a commercial assay kit and the measurements were made on the spACE analyser (Alfa Wassermann, Inc., West Caldwell, NJ, USA). Fibrinogen concentration was measured using a commercial assay kit (Boehringer Mannheim, Germany). Glucose, globulin and aspartate amino transferase (AST) concentrations in plasma were determined using a commercial kit (Randox Laboratories Ltd., Crumlin, Antrim, N. Ireland; catalogue No. GL2623) on an automated analyzer (Hitachi 705, Boehringer Mannheim, Lewes, East Sussex, UK). All other physiological measurements were made using Randox assay procedures.

Environmental conditions

The transporter was fitted with sensors for measuring ambient temperature (°C), relative humidity (RH), carbon dioxide concentration (mg/kg), air velocity (m/s) and vapour density (g/m^3) continuously during transport. The ambient temperature and relative humidity during transport and in the housing environment were recorded continuously using TinyTalk dataloggers (Radionics, Dublin, Ireland). Relative humidity, CO_2 concentration and wind velocity were measured using Testo 445 portable multifunction probes (Testo UK, Ltd).

Body temperature

The rectal and body surface (neck, rump and tail areas) temperatures were monitored before and after transport from the T and C bulls using a digital electronic thermometer (Jorgen Kruuse A/S; Model VT-801BWC Lot No. 0701) and laser device (Raynger MX4, Infrared laser thermometer, Raytek, Radir Ltd, Bletchley, Milton Keynes, UK), respectively. All animals were measured prior to transport (day 0) and again on days 2, 3, 5, 7, 9, 11 and 38 of the study.

Statistical analysis

Data were analysed using SAS/STAT for windows (SAS, 1996). Data on albumin, AST, BHB, glucose, NEFA, LDH, percentage and number of neutrophils, percentage and number of lymphocytes, haematocrit, cortisol, and WBC were subjected to a one-way ANOVA by Proc GLM, using a means statement with the Tukey option to evaluate treatment differences. A matched-pair t-test was used to assess differences between pre- and post-transport for each treatment. Live-weight data and rectal and surface temperature measurements were analysed using the repeated measures procedure in Proc GLM while controlling for time effects. Measurements for fibrinogen, Hb, RBC, and Con–A- and KLH-induced IFN-γ production were not normally distributed. They were analysed by Proc GLM, using ranked data in a Kruskall-Wallis test with a Tukey option to evaluate treatment differences. A Wilcoxon signed rank test was used to evaluate differences between pre- and post-transport for each treatment (Snedecor and Cochran, 1989).

Results

Environmental conditions

The relative humidity recorded in the transporter ranged from 64.4 to 90.7% mean

(s.d.) 80.7 (0.90) %), the vapour density ranged from 8.1 to 13.2 g/m^3 (mean (s.d.) 10.9 (0.15) g/m^3), and the temperature (^{o}C) in the transporter ranged from 10.0 to 18.9 ^{o}C (mean (s.d.) 14.2 (0.27) ^{o}C). The RH in the housing environment for control animals ranged from 59.5 to 88.0% (mean (s.d.) 74.7 (0.92)) and the temperature ranged from 11.0 to 18.5 ^{o}C (mean (s.d.) 15.1 (0.22) ^{o}C). The outside ambient relative humidity ranged from 45.6 to 81.5% (mean (s.d.) 56.6 (0.884) %) and the temperature ranged from 9.9 to 17.0 ^{o}C (mean (s.d.) 13.5 (0.25) ^{o}C). Carbon dioxide concentrations were recorded during transit and ranged from 334 to 1,138 mg/kg (mean (s.d.) 619 (18.9) mg/kg). The wind velocity during transport ranged from 0.06 to 2.09 m/s, (mean (s.d.) 0.4 (0.30) m/s).

Body temperature

There were no differences among treatment groups in mean rectal body temperature, pre- or post-transport. However, all rectal temperatures were greater ($P \leq 0.05$) prior to transport than following transport (Control; mean (s.d.) ^{o}C were: 38.6 (0.33) vs. 38.5 (0.32); T085; 39.0 (0.42) vs. 38.4 (0.42); T127; 38.9 (0.41) vs. 38.1 (0.53)).

Live weight

There were no significant differences among treatment groups before or after the 12-h transport period. Mean (s.d.) live weights pre- versus post-transport were 249.2 (18.77) vs. 249.5 (18.96), 249.8 (20.72) vs. 249.1 (20.25) and 250.2 (43.38) vs. 254.6 (43.06) for Control, T085 and T127, respectively.

Physiological, haematological and immunological variables

There were no significant differences in monocyte number, percentage monocytes, platelet number, MCH, MCHC, MCV, lactate, protein, globulin or urea following transportation.

Albumin: There were no differences among treatments in albumin concentration prior to transport (Table 1). Following transportation both transported groups had significantly greater albumin concentration than the control group. Bulls transported at the 0.85 m^2 spatial allowance had significantly higher concentrations ($P \leq 0.05$) than pre-transport. Control animals had significantly lower concentrations post-transport ($P \leq 0.0001$) than the pre-transport baseline.

Aspartate amino transferase: There were no differences ($P \geq 0.1$) among treatment groups in AST concentration before or after transport (Table 1). However, AST concentrations were significantly greater ($P \leq 0.001$) in both control and transported animals compared with baseline values.

Betahydroxybutyrate: There were no differences among treatment groups in mean BHB concentrations prior to transport (Table 1). BHB concentrations were significantly lower in the two transport groups post-transport compared with control and BHB concentrations were lower ($P \leq 0.001$) in all groups than the pre-transport concentrations.

Glucose: There were no differences in glucose concentration among treatments prior to transport (Table 1). Following transport, blood glucose concentrations were greater ($P \leq 0.001$) in animals transported at 0.85 m^2 and 1.27 m^2 than in controls.

Non-esterified fatty acids: Pre-transport NEFA concentration did not differ among treatment groups. Following transport, animals transported at a spatial allowance of 1.27 m^2 had a significantly higher NEFA concentration than control animals (Table 1).

Lactate dehydrogenase: LDH activity was lower ($P \leq 0.05$) in both transported groups

Table 1. Treatment means (± s.d.) for various blood constituents pre- and post-transport

Variable[2]	Treatment[1]	Pre-transport	Post-transport
Albumin (g/L)	Control	$32.8^{a} \pm 1.75$	$31.1^{bx} \pm 1.55$
	T127	$33.6^{a} \pm 1.74$	$33.9^{ay} \pm 1.78$
	T085	$33^{a} \pm 1.7$	$33.7^{by} \pm 1.29$
Aspartate amino transferase (AST) (U/L)	Control	$62.9^{a} \pm 8.0$	$73.1^{b} \pm 11.78$
	T127	$65.2^{a} \pm 9.26$	$75.8^{b} \pm 18.95$
	T085	$61.9^{a} \pm 9.63$	$74.6^{b} \pm 13.97$
Betahydroxy-butyrate (BHB) (g/L)	Control	$0.39^{a} \pm 0.084$	$0.25^{bx} \pm 0.068$
	T127	$0.41^{a} \pm 0.076$	$0.15^{by} \pm 0.071$
	T085	$0.43^{a} \pm 0.109$	$0.17^{by} \pm 0.054$
Glucose (mmol/L)	Control	$4.16^{a} \pm 0.528$	$4.21^{ax} \pm 0.279$
	T127	$4.26^{a} \pm 0.331$	$4.79^{by} \pm 0.452$
	T085	$4.16^{a} \pm 0.283$	$5.01^{by} \pm 0.405$
Non-esterified fatty acids (NEFA) (μmol/L)	Control	0.135^{a} (0.11–0.16)	0.15^{ax} (0.08–0.22)
	T127	0.13^{a} (0.11–0.17)	0.29^{by} (0.19–0.56)
	T085	0.135^{a} (0.09–0.39)	0.295^{by} (0.21–0.42)
Lactate dehydrogenase (LDH) (U/L)	Control	1709.5^{xa} (1442–2432)	1786.0^{a} (1424–2304)
	T127	2204.0^{ya} (1697–2878)	1856.0^{b} (1608–2183)
	T085	2236.0^{ya} (1467–3016)	1680.0^{b} (1479–2578)

[1]Control = not transported; T127 = transported for 12 h at a stocking density of 1.27 m^2 per animal; T085 = transported for 12 h at a stocking density of 0.85 m^2 per animal.

[2]Values are expressed as mean ± s.d. for albumin, AST, BHB, glucose and as median with minimum and maximum values for NEFA and LDH.

[a,b]Within row means not having a common superscript differ significantly ($P \leq 0.001$).

[x,y]Within column means not having a common superscript differ significantly ($P \leq 0.001$).

than their pre-transport baseline values but were not different for controls. Pre-transport concentrations were greater ($P \leq 0.001$) in the animals that were assigned to the 0.85 and 1.27 m^2 spatial allowance treatments (Table 3) compared with controls.

Lymphocyte and neutrophil: The lymphocyte percentage and lymphocyte number were reduced ($P \leq 0.001$) in the transported animals post-transport (Table 2). The neutrophil percentage and the number of neutrophils were greater ($P \leq 0.001$) in the transported animals than in controls (Table 2).

Haematocrit: There were no differences in haematocrit percentage among treatment groups prior to transport (Table 2). Haematocrit was significantly higher in all treatment groups post-transport when the blood concentrations were compared with pre-transport values. However, haematocrit for the animals transported at 1.27 m^2 was greater ($P \leq 0.001$) than the controls.

Red blood cell numbers

There were no differences in RBC number among treatment groups before transport (Table 2). RBC numbers post-transport were higher in the animals transported at a spatial allowance of 1.27 m^2 than controls, however, the numbers were within normal referenced ranges.

White blood cells

There were no differences in WBC number among treatment groups before transport (Table 2). WBC numbers post-transport were higher in the animals

Table 2. Treatment means (± s.d.) for various blood cell types and percentages prior to transport and after a 12-h journey

Variable[2]	Treatment[1]	Pre-transport	Post-transport
Lymphocytes (%)	Control	$56.4^{a} \pm 12.14$	$57.5^{ax} \pm 10.93$
	T127	$57.3^{a} \pm 6.59$	$44.7^{by} \pm 8.84$
	T085	$58.1^{a} \pm 8.13$	$40.8^{by} \pm 10.13$
Lymphocyte number	Control	$5.9^{a} \pm 1.04$	$5.9^{a} \pm 1.35$
	T127	$6.0^{a} \pm 1.28$	$5.0^{b} \pm 1.10$
	T085	$6.3^{a} \pm 1.02$	$5.2^{b} \pm 1.61$
Neutrophils (%)	Control	$39.9^{a} \pm 11.09$	$38.4^{ax} \pm 10.91$
	T127	$38.4^{a} \pm 6.83$	$51.3^{by} \pm 9.33$
	T085	$38.2^{a} \pm 8.0$	$55.6^{by} \pm 10.05$
Neutrophil number	Control	$4.5^{a} \pm 2.06$	$4.1^{ax} \pm 1.89$
	T127	$4.0^{a} \pm 1.05$	$6.0^{by} \pm 1.66$
	T085	$4.3^{a} \pm 1.5$	$7.1^{by} \pm 2.55$
Haematocrit (%)	Control	$29.1^{a} \pm 3.24$	$29.7^{bx} \pm 3.29$
	T127	$31.8^{a} \pm 2.6$	$33.2^{by} \pm 2.99$
	T085	$30.9^{a} \pm 2.6$	$32.5^{bxy} \pm 3.46$
Red blood cell number ($\times 10^6/\mu L$)	Control	8.36^{a} (4.66–9.74)	8.53^{bx} (4.65–9.58)
	T127	9.32^{a} (5.59–10.6)	9.6^{by} (5.78–11.8)
	T085	8.52^{a} (6.25–10.5)	9.05^{bxy} (6.68–11.9)
White blood cell number ($\times 10^3/\mu L$)	Control	$10.9^{a} \pm 2.4$	$10.4^{ax} \pm 2.27$
	T127	$10.5^{a} \pm 2.02$	$11.7^{ay} \pm 2.53$
	T085	$10.9^{a} \pm 2.13$	$12.5^{axy} \pm 2.64$

[1]Control = not transported; T127 = transported for 12 h at a stocking density of 1.27 m^2 per animal; T085 = transported for 12 h at a stocking density of 0.85 m^2 per animal;
[2]Values are expressed as mean ± s.d. except for red blood cell number which is given as median with range in parentheses.
[a,b]Within row means not having a common superscript differ significantly ($P \leq 0.001$).
[x,y]Within column means not having a common superscript differ significantly ($P \leq 0.001$).

transported at a spatial allowance of 0.85 m^2 than controls, however, the numbers were within normal referenced ranges.

Haemoglobin: Haemoglobin concentration was greater ($P \leq 0.05$) in the 1.27 m^2 treatment group prior to transport. Following transport, haemoglobin concentration for the animals transported at 1.27 m^2 was greater than controls (Table 3).

Creatine kinase: There were no significant differences among treatments in CPK concentration before or after transport (Table 3).

Acute phase proteins: There were no differences in fibrinogen concentration among treatment groups before transport (Table 3). Following transport, plasma fibrinogen concentration was lower ($P \leq 0.001$) in the two transported groups than controls (Table 3).There were no differences in haptoglobin concentration among treatment groups before transport (Table 3). Following transport, plasma haptoglobin concentration was lower ($P \leq 0.001$) in the two transported groups than controls (Table 3).

Cortisol: The data for plasma cortisol concentration are presented in the Table 4. Basal plasma cortisol concentration was greater ($P \leq 0.05$) for T085 compared

Table 3. Treatment values for creatine kinase, fibrinogen, haptoglobin and in plasma and concentration of haemoglobin in whole blood prior to transport and after a 12-h journey

Variable[2]	Treatment[1]	Pre-transport	Post-transport
Creatine kinase (μmol/L)	Control	131.5^{a} (104–384)	146^{a} (105–232)
	T127	142^{a} (100–204)	161^{a} (109–206)
	T085	156.5^{a} (95–226)	160.5^{a} (125–986)
Fibrinogen (mg/dl)	Control	553.0^{a} (441–723)	646.5^{bx} (527–968)
	T127	535.0^{a} (459–771)	549.0^{ay} (439–728)
	T085	510.0^{a} (449–1,265)	530.0^{ay} (481–1,546)
Haptoglobin	Control	0.220^{a} (0.15–1.09)	0.190^{a} (0.14–1.54)
(Hb binding capacity/L)	T127	0.200^{a} (0.16–0.34)	0.250^{a} (0.17–0.36)
	T085	0.215^{a} (0.16–3.02)	0.215^{a} (0.17–2.45)
Hb (gm/100dl)	Control	10.3^{w} (6.9–11.5)	10.45^{bx} (6.9–11.6)
	T127	11.3^{z} (9.4–12.3)	11.5^{by} (10–12.9)
	T085	10.8^{wz} (9.1–12.4)	11.3^{bx} (9.6–13.3)

[1]Control = not transported; T127 = transported for 12 h at a stocking density of 1.27 m ^{2} per animal; T085 = transported for 12 h at a stocking density of 0.85 m^{2} per animal.
[2]Values are expressed as median with minimum and maximum values in parentheses.
[a,b]Within row means not having a common superscript differ significantly ($P \leq 0.001$).
[x,y]Within column means not having a common superscript differ significantly ($P \leq 0.001$).

with control but were not different from the T127 group. There were no significant treatment differences post-transport.

Interferon-γ production: There were no differences among treatments in IFN-γ production, in response to *in vitro* stimulation of whole blood cultures incubated with either Con-A or keyhole limpet haemocyanin, either before or after transport (Table 4).

Discussion

The results from this study show that bulls undergoing 12-h transportation by road at spatial allowances of 0.85 m^{2} or 1.27 m^{2} showed physiological, haematological and immunological responses that were within normal referenced ranges (Schalm, 1961, 1984; Kaneko, 1989).

Acute elevation of plasma glucocorticoids (cortisol), from activation of the hypothalamic-pituitary-adrenal (HPA) axis, and suppression of immune function (suppression of lymphocyte blastogenesis (Murata *et al*., 1987; Murata, 1997) and IFN-γ production (Earley and Crowe, 2002; Ting, Earley and Crowe, 2003a; 2003b; 2004), are key defining features of stress response in cattle. In the present study, it may be hypothesised that the 12-h transport did not induce sufficient stress to induce changes in the activity of the pituitary-adrenal axis. Glucocorticoid secretion plays a major role in the stress-induced suppression of immune-inflammatory reactions (Webster, Tonelli and Sternberg, 2002). Studies designed to evaluate the acute effects of transport, including loading and journey length, on calf welfare (Knowles *et al.,* 1993; 1997; Murata, Takahashi and Matsumoto, 1987; Blecha, Boyles and Reilly, 1984; Todd *et al*., 2000; Mormede *et al.*, 1982; Staples and Haugse, 1974) have shown a transient acute physiological response to transport and handling (characterised by increased plasma cortisol concentration) along with other biological responses that are related mainly to feed and water restriction

Table 4. Treatment means (± s.d.) for plasma cortisol concentration and for interferon-γ production by cultured lymphocytes following induction by either concavalin A (Con-A) or keyhole limpet hemocyanin (KLH) prior to transport and after 12-h journey

Variable	Treatment[2]	Pre-transport	Post-transport
Cortisol (ng/mL)	Control	7.48 ± 6.91^{ax}	6.91 ± 4.44^{a}
	T127	8.17 ± 5.22^{axy}	7.09 ± 4.80^{a}
	T085	9.15 ± 4.64^{ay}	7.94 ± 3.00^{a}
Con-A IFN-γ[1]	Control	0.278 ± 0.20^{a}	0.224 ± 0.179^{a}
	T127	0.230 ± 0.216^{a}	0.189 ± 0.158^{a}
	T085	0.197 ± 0.169^{a}	0.181 ± 0.190^{a}
KLH IFN-γ[1]	Control	0.026 ± 0.046^{a}	0.029 ± 0.047^{a}
	T127	0.024 ± 0.037^{a}	0.009 ± 0.035^{a}
	T085	0.021 ± 0.031^{a}	0.012 ± 0.048^{a}

[1]Expressed as optical density measured at 450 nm.
[2]Control = not transported; T127 = transported for 12 h at a stocking density of 1.27 m^2 per animal; T085 = transported for 12 h at a stocking density of 0.85 m^2 per animal.
[a,b]Within row means not having a common superscript differ significantly ($P \leq 0.001$).
[x,y]Within column means not having a common superscript differ significantly ($P \leq 0.001$).

during transport. In the present study, plasma cortisol concentrations were unchanged following the 12-h transport journey. It is likely, however, that if animals were blood sampled during transit, that acute elevations in cortisol would have been observed within the first 1 to 2 h of transport. However, the techniques necessary for non-invasive blood sampling during transit need to be developed and further investigation of changes in plasma cortisol during transport is warranted.

In this study, there was no significant change in IFN-γ production in response to either Con-A or KLH stimulation of whole blood cells, indicating that there was no significant change in immunological response of bulls post-transport. Measurement of *in vitro* IFN-γ production in whole blood cultures gives a sensitive indication of cell-mediated immunity (Rothel *et al.*, 1992), and the method has been well established in the bovine castration model (Fisher *et al.*, 1997a, 1997b; Earley and Crowe, 2002). Reduced lymphocyte blastogenic response in calves (Blecha *et al*., 1984; Murata *et al*., 1987) and suppressed natural killer-cell activity in pigs (McGlone *et al*., 1993) have been reported following transport. Moreover, Blecha *et al*. (1984) transported steers (180 to 280 kg live weight) for 10 h by road and showed that blood neutrophil numbers were increased, lymphocyte blastogenic response was suppressed, and these effects were independent of feed and water deprivation.

Blood cell constituents are sensitive indicators of the physiological or pathophysiological responses of animals to stress (Radostits, Blood and Gay, 1994). Changes in the populations of white blood cell types in response to stressors, particularly the relative decrease in lymphocyte compared with neutrophil numbers, have been measured in studies relevant to animal welfare but have not always been correlated with increased susceptibility to disease. In the present study, the percentage lymphocyte and lymphocyte numbers were reduced in the transported animals post-transport, while the percentage of neutrophils and the number of neutrophils increased significantly in all transported

animals. Increased neutrophil, decreased lymphocyte and eosinophil percentages have been reported following handling in cattle (Murata *et al*., 1987) and with diseases of bacterial origin (Radostits *et al*., 1994). Neutrophilia and lymphopenia are common findings in stressed animals and are associated with changes in WBC trafficking and release from the bone marrow by elevated concentrations of glucocorticoids (Dunn, 1989), this is contrary to the absence of change in plasma cortisol in the present study.

The haematocrit values and red blood cell numbers were significantly higher in the transported bulls in the present study, indicating a degree of dehydration. However, haematocrit for the animals transported at 1.27 m^2 was greater than the controls. The findings that the concentration of haptoglobin was unchanged following transportation while plasma fibrinogen concentrations were significantly reduced in the two transported groups were unexpected as inflammatory responses are normally associated with raised fibrinogen levels.

The absence of any change in the activity of creatine kinase suggests that the journey was not physically stressful. The reasons for increased plasma glucose following transport may reflect increased plasma catecholamine secretion causing an increase in glycogen metabolism. In a study with young calves, Kent and Ewbank (1986), transported three-month-old calves for either 6 or 18 h and showed that plasma glucose concentrations were elevated in transported calves compared with starved controls, as were plasma cortisol, non-esterified fatty acids and total white blood cell numbers.

Eldridge, Winfield and Cahill (1988) transported heifers (350 kg live weight) at space allowances of either 0.89 to 0.9 m^2/animal or 1.10 to 1.14 m^2/animal over journeys of differing duration. Heifers transported at the lower space allowance had lower heart rate and movement scores, and it was postulated that transport of cattle in vehicles with small pens at small space allowances was preferable because there was more support against involuntary movement. This is in direct contrast to the conclusions of Tarrant, Kelly and Harrington (1988) and Tarrant *et al*. (1992). Tarrant *et al*. (1988) transported steers (603 kg live weight) at space allowances of 1.02, 1.93 and 3.00 m^2/animal while Tarrant *et al*. (1992) use allowances in the range 1.03 to 1.4 m^2/animal. Plasma cortisol, creatine kinase, muscle bruising observed at slaughter, and the incidence of animals going down during transport and being unable to rise, all increased with decreasing space allowance. Grigor *et al*. (2001) investigated the effects of space allowance during transportation (two 9-h journeys) and duration of a mid-journey lairage period (1 or 12 h between journeys) on measurements of stress, injury, dehydration, food restriction and rest in young calves. The authors concluded that there was little evidence that transport affected immunological variables, but there was some evidence that the health of calves post-transport was affected.

It is concluded that, within the conditions of the present study, there was no significant biological effects on the variables studied when transporting bulls at a space allowance of 1.27 m^2/animal compared with a spatial allowance of 0.85 m^2 on a 12-h road journey.

Acknowledgments

The authors gratefully acknowledge the technical assistance of: Francis Collier, Martin Donlon, J.A. Farrell, Sandeep Gupta, Joe Larkin, Paddy Mallon, Ann Marley, Mary Munnelly, Margaret Murray, Joe Munroe, Liam Moore, Michael Nolan, Julianne

Price, Dan Prendiville and Simon Perry. Many thanks are due to: the farm foreman, Gerry Santry, farm staff – Gabriel Costelloe, Joe Gill, Dave Gorman, Frank Gorman, John Horan, Eddie Mulligan, Hugh Mulligan, Paschal Reilly, Martin Ryan, for their assistance throughout the experiment; to Ann Gilsenan, for typesetting and graphics; to Paddy Guernan for the articulated transporter.

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Received 21 June 2004