Occasional Report 4
ISBN 1 84170 307 9

The welfare of animals transported from Ireland to Spain
AND
The physiological, haematological and immunological responses of 9-month old bulls (250kg) to transport at two stocking densities (0.85m^2 and 1.27m^2 /250kg animal) on a 12-hour journey by road.

Authors

Bernadette Earley, Joseph A. Farrell, Margaret Murray, Dan Prendiville, Edward G. O'Riordan

2003

Teagasc
Grange Research Centre
Dunsany
Co. Meath
Ireland

ISBN 1 84170 307 9
Grange Research Centre

Experiment 1:

The welfare of animals transported from Ireland to Spain

Occasional Report 4
ISBN 1 84170 307 9

Bernadette Earley, Joseph A. Farrell, Margaret Murray,
Dan Prendiville, Edward G. O'Riordan

2003

2003

2003

Teagasc
Grange Research Centre
Dunsany
Co. Meath
Ireland

ISBN 1 84170 307 9
Grange Research Centre

Contents **Page No**

Experiment 1:
The welfare of animals transported from Ireland to Spain

1. Summary

Fifty-two weanling continental x beef heifers (mean liveweight 269kg) were transported from Ireland to France on a roll-on roll-off ferry (RO-RO), and onwards by road for 3-hours to a French lairage, rested for 24 hours at a staging post and taken by road on an 18-hour journey through France to a feedlot in Spain. Animals transported to France lost 7.6 % of their bodyweight, and gained 3.3 % of their bodyweight by time of arrival in Spain and recovered to pre-transport liveweight values by day 6. Although there was some evidence that transport affected physiological and immunological variables, there was no evidence to suggest that it adversely affected the health or the performance of the animals post transport.

Creatine kinase activities were increased but values were still within normal acceptable ranges. Increases in non-esterified fatty acids, βeta-hydroxybutyrate and urea concentrations suggested that the animals' normal pattern of feeding was disrupted during transport. Increases in albumin, total plasma protein and osmolality would indicate slight dehydration during transit. However, albumin concentrations returned to control levels by day 38 of the study. While haematocrit values were decreased, they are within the range of normal referenced data (24 - 48%). Similarly, changes in the RBC numbers and haemoglobin were within the normal blood referenced ranges ((RBC; 5.0 – 10.0 $x10^6$ /μl) and (haemoglobin 8-14 g%)(Schalm, 1961)). The only time at which white blood counts increased above the upper limit of 12, was 12 hours after arrival at the French lairage. The aspartate transaminase concentrations for the transported animals at arrival in France and Spain were not significantly different from their pre-transport concentrations but were increased at day 11 when compared with baseline levels.

Concanavalin-A induced interferon-γ levels were lower on arrival in the Spanish feedlot and on Day 11 of the study, when compared with pre-transport baseline levels. Compared with pre-transport levels, keyhole limpet haemocyanin-induced interferon-γ levels for the transported animals were significantly decreased on the day of arrival in France, with no significant difference on the day of arrival in Spain or on day 11 of the study. Interferon-γ is produced by activated T lymphocytes and natural killer cells in response to antigen. The percentage (%) of lymphocytes decreased and the % neutrophils increased post-transport indicating a shift in the population of these blood cells relative to pre-transport baseline values. There was no significant change in plasma cortisol concentrations in transported animals at arrival in France and in Spain. On Day 11, the plasma cortisol concentrations of transported animals were significantly higher than control animals.

There were significantly higher glucose concentrations on arrival in France, and in samples taken at 12 and 24 hours post-arrival in France, on arrival in Spain, and on days 7 and 11 compared with control levels. Transported animals had significantly higher glucose levels at sample 2 on the day of arrival in France compared with their pre-transport values.

Transported animals had significantly higher fibrinogen levels at arrival in France compared with their pre-transport baseline concentrations. Inflammation resulting from stress can cause the release of acute phase proteins such as haptoglobin and fibrinogen, and acute phase proteins in cattle have been associated with immunosuppression, however, much higher levels have been reported in inflammatory conditions. Transported animals had significantly higher non-esterified fatty acid (NEFA) levels on arrival in France and Spain and on day 11 compared with their pre-transport baseline concentrations. Control animals had significantly higher levels on day 5 compared with their pre-transport baseline NEFA concentrations. However, all levels were within the normal acceptable ranges.

The study concluded that transport had no adverse effect on animal welfare based on the physiological, immunological and haematological measurements made.

2. Introduction

The protection of animals during transport is an important concern of the European Commission. The first Community legislation on the protection of animals during transport, Council Directive 77/489/EC, reflected the relevant 1968 Convention of the Council of Europe. It has since been replaced by the more detailed Council Directive 91/628/EC as amended by Directive 95/29/EC which introduced changes such as the approval of transporters, route plan, as well as loading densities and travelling times limit. Additional legislation reinforcing Directive 91/628/EEC was adopted in 1995, 1997 and 1998. Transportation of livestock is perceived as an acute stressor and involves several potential stressors that result in increase cortisol (Kenny and Tarrant, 1987a, b), altered products of energy and protein metabolism (Todd *et al.* (2000), with associated changes in appetite and growth rate and a challenged immune system (Blecha *et al.*, 1984; Murata *et al.*, 1987) resulting in increased disease susceptibility. Other physical factors such as noise or vibrations; emotional factors, such as unfamiliar environment or social regrouping; and climatic factors, such as temperature, humidity, or oxygen concentration, are also involved.

The overall objective of the present study was to investigate the physiological, haematological and immunological responses of weanling heifers transported under present EU legislation and to evaluate the implications in terms of animal welfare.

3. Objectives

1. To make appropriate physiological measurements on the animals to quantify the effect of transport on the degree of stress imposed and the ability of the animals to cope with that stress.
2. To monitor and record the environmental conditions on the vehicle (as normal) thus enabling the heat and moisture production of the animals to be determined.

Study hypothesis: the welfare of animals transported from Ireland to Spain will not be compromised in transit or subsequently as a result of the journey.

4. Materials and methods

Fifty-two weanling continental x beef suckler heifers (mean ± sem liveweight 269.1 ± 6.33kg), sourced from 10 different beef suckler farms in Co's Cavan and Longford, having been weaned between October 2nd and December 8th, were transported by road and sea to Spain. On the morning of the journey (December 8th, 2001), 52 animals were blood sampled (day 0; Sample 1) by jugular venepuncture to provide baseline physiological values on the farm of origin. Fifty-two animals were then taken to a local mart in Co. Cavan, weighed and randomly allocated, at 18:00h, into 6 naturally ventilated and 6 fan ventilated pens at a stocking density of $0.9m^2$ per animal, on an air suspension double deck articulated transporter and transported by road (231km) to Co. Wexford. The animals were unloaded and lairaged, feed and water was availability overnight in Co. Wexford. The animals were loaded on the transporter into the same pens on December 9th and transported by road (31km) to the ferry port at Rosslare. The pens on the transporter were bedded with straw and water was available through nipple drinkers. The ferry departed Rosslare at 17:00hr and the journey took approximately 23hours. The average speed during the sailing ranged from 14 to 14.5 knots/hr, the wind/force ranged from SE/5 – SE/6, and the ambient temperature ranged from 8 to 11 ^{o}C. Twenty-eight weanling continental x beef breed heifers (282.5 ± 8.96kg liveweight) were weaned at the same time as the transported animals and remained on two control farms (N = 16 and N =12 per farm) and were blood sampled and weighed at times corresponding to the transported animals.

On arrival in Cherbourg, France on December 10th at 15:45h, the animals were transported by roadfor 3h to a lairage in Fougeres, where they remained for 24 hours. At the lairage, animals were unloaded, and weighed. They were blood samples immediately on arrival (Day 2; sample 2), and again at 12 hours (Day 3; sample 3) and 24 hours (Day 3; sample 4) after arrival in the lairage by jugular venepuncture into blood collection tubes containing anticoagulant (see Table 1 with experimental protocol of study and dates of blood sampling). Hay and water were freely available in the lairage.

Table 1: Experimental protocol for the transport study from Ireland to Spain.

	Pre-transport	**Departure (Ferry)**	**Arrival in French lairage**	**Lairage**	**Lairage Depart for Spain**	**Arrival in Spanish Feedlot**	**Feedlot**				
	Ireland	**Ireland to France**	**France time 0**	**France +12 hr**	**France +24 hr**	**Spain**	**Spain**	**Spain**	**Spain**	**Spain**	**Spain**
Date	08-Dec	09 Dec	10-Dec	11-Dec	11-Dec	12-Dec	15-Dec	17-Dec	19-Dec	15-Jan	28-Feb
Day of study	**0**	1	**2**	3	3	**5**	7	9	**11**	**38**	82
Sample No.	**1**		**2**	3	4	**5**	6	7	**8**	**9**	
	Live weight		Live weight			Live weight			Live weight	Live weight	Live weight

The 18 hour journey from the lairage at Fougeres in France to the feedlot in Fuensalida, Spain (1300km) involved different road surfaces ranging from motorways to country lanes. To comply with current legislation, animals were rested for one hour on the transporter after the first 14 hours of the journey. On arrival in the Spanish feedlot (December 12th) on Day 5, animals were blood sampled (sample 5) and again on days 7, (sample 6), 9 (sample 7) 11 (sample 8) and 38 (sample 9) of the study. The animals were weighed after unloading at the feedlot in Spain, and on day 11 and 38 of the study. A final liveweight was taken on the 28th of February (day 82 of the study).

The animals in Spain were fed an *ad libitum* finishing concentrate diet (DM 887g/kg; Crude protein 155 g/kg; Ash 58.6 g/kg; crude fibre 41.9 g/kg; Oil b 39.0 g/kg ADF 53.6g/kg; NDF 157 g/kg; Oil ME 39) and straw (DM 907g/kg; Crude protein 44 g/kg; Ash 74.1 g/kg; Crude fibre 303g/kg; Oil B 8.8 g/kg; ADF 348 g/kg; NDF 630 g/kg; Oil ME 6.8. The control animals remaining on the farms in Ireland were maintained on an *ad libitum* silage diet and concentrates (2kg/head).

Rectal temperatures were recorded using a digital thermometer (Jorgen Kruuse A/S; Model VT-801BWC Lot No 0701) prior to transportation (day 0) and on days 2, 3, 5, 7, 9, 11 and 38 of the study.

5. Physiological, haematological and immunological variables.

Blood samples (Samples 1..9) were collected by jugular venepuncture, into heparinised tubes, centrifuged and the plasma separated for subsequent analysis of cortisol, glucose, lactate, free fatty acids, beta-hydroxy butyrate, urea, total protein, albumin, creatine phosphokinase (CK), lactate dehydrogenase (LDH), and the acute phase proteins (fibrinogen and haptoglobin). Blood samples for interferon-γ determination were also collected by jugular venepuncture into aseptic vacutainer tubes containing lithium heparin and the stimulated lymphocyte production of interferon-γ in response to keyhole limpet haemocyanin (KLH) and Concanavalin-A (Con-A), was determined following whole blood culture of heparinised samples, using an ELISA procedure (CSL, Biosciences, Parkville, Victoria, Australia).

The haematological variables (red blood cell number (RBC), haemoglobin (Hb), haematocrit % (or packed cell volume) (PCV), total white blood cell (WBC) numbers, % lymphocytes, % monocytes) were determined in unclotted (K_3-EDTA) whole blood samples using an electronic particle hematology analysers (CellDyn 3500 Analyser (Ireland), Technicon H1, manufactured by Bayer (Spain)**,** Cherpy-Gaillot French laboratory, PENTRA500 apparatus (ABX company). Plasma cortisol concentrations were determined using a commercially available radioimmunoassay (RIA) kit. Plasma haptoglobin concentrations were measured by determining the haemoglobin-binding capacity using a biochemical autoanalyser. Fibrinogen concentrations were measured using a commercial biochemical assay kit (Boehringer Mannheim, Germany). All other physiological measurements were made using Randox assay procedures. Bodyweight, rectal temperature and blood haematology (WBC numbers) were measured as general indicators of health and the ability of animals to cope with transport.
Temperature, relative humidity during transport were recorded using TinyTalk Ultra dataloggers (UK). On each deck, monitoring of environmental conditions were made at Bay 5 (rear), Bay 3 (middle) and Bay 1 (front). [i.e. Bay 1 was the first pen loaded on each deck at the front, and Bay 5 was the last pen loaded.

6. Statistical analysis

The physiological, haematological and physiological measurements taken before and after transport were analysed by repeated measures analysis of variance with journey time as the factor. The first sample (Day 0; sample 1) was used as a covariate in this analysis. SAS/STATISTIC® software was used to analyse the data for the study. Pre-planned, matched pair t-tests, to detect changes over time were made using PROC MEANS, the null hypothesis being that the mean difference between selected time points was equal to zero. The PROC GLM repeated measures option was used to test the effects of treatment while controlling for time effects. Analysis was performed on the rank scores of variables that failed the test for normality.

7. Results and Discussion

7.1 Environmental conditions

7.1.1 Temperature

The graphs summarises the variation in ambient temperature (Figure 1) and vapour density (Figure 2) for the lower and upper decks. In essence the data points show the trends along the length, at mid-line, of the vehicle.

Although the lower deck was mechanically ventilated, the logistics of switching power supplies at boarding and disembarking the ferry meant that the lower deck was switched to a naturally ventilated configuration - to match the upper deck. As landing was scheduled for 15:30, the system was changed over at 14:30, after which time the mechanically ventilated deck became naturally ventilated. This changeover explains the sudden rise in temperatures for the lower deck.

The notable points on the temperature plots are:

1. Ambient values rose from around 12°C on sailing to about 15°C for the rest of the crossing. On disembarkation in Cherbourg, ambient temperature dropped from 15°C to 2°C. Ambient was still low on leaving Fougeres (overnight) and actually went below freezing on crossing into Spain.
2. Generally, on the lower mechanically ventilated deck the expected profile (from rear inlet to front outlet) was maintained (on the ferry and for the duration of the transportation by road transport into Spain.
3. There were marked differences on the upper (naturally ventilated) deck where temperatures were higher on the ferry and lower during the transit into Spain. These might suggest inadequate ventilation on the ferry and excessive ventilation on the road.

The minimum and maximum temperatures for the feedlot were –5.3^{o}C (December 16th) and 13.8^{o}C (January 14th) and the respective values for the relative humidity were 25.3% and 100%.

Figure 1: Variation in temperature during transport

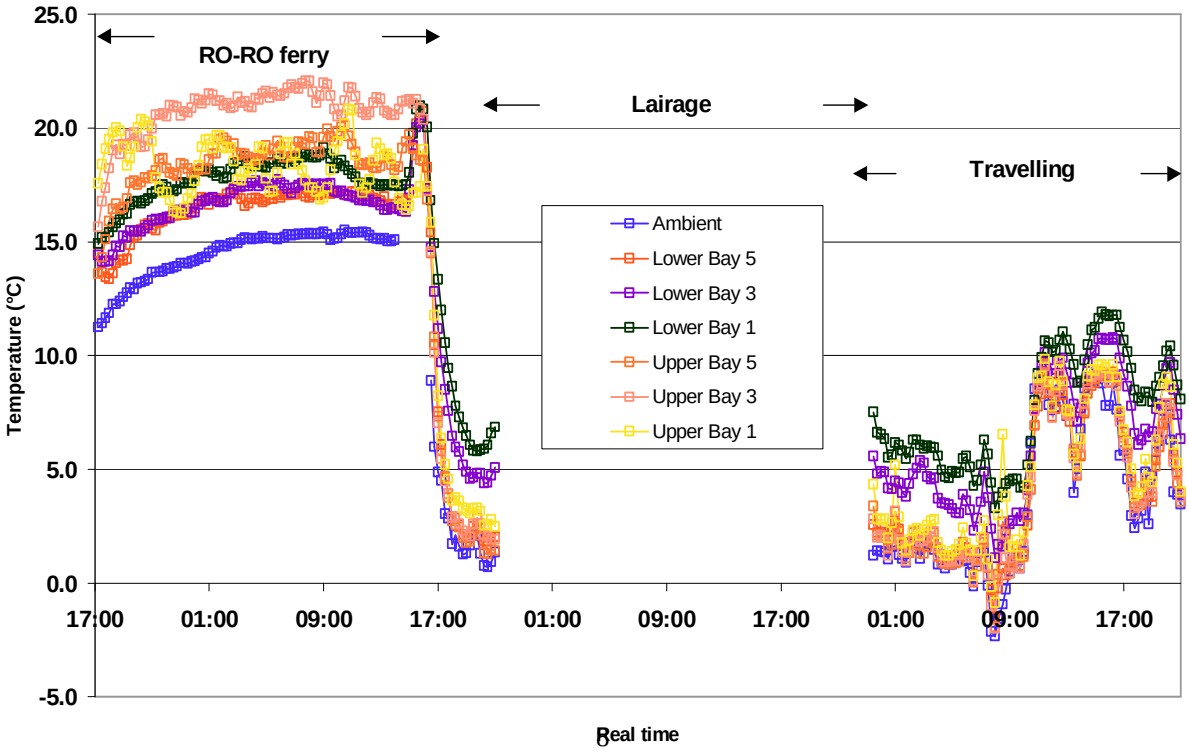
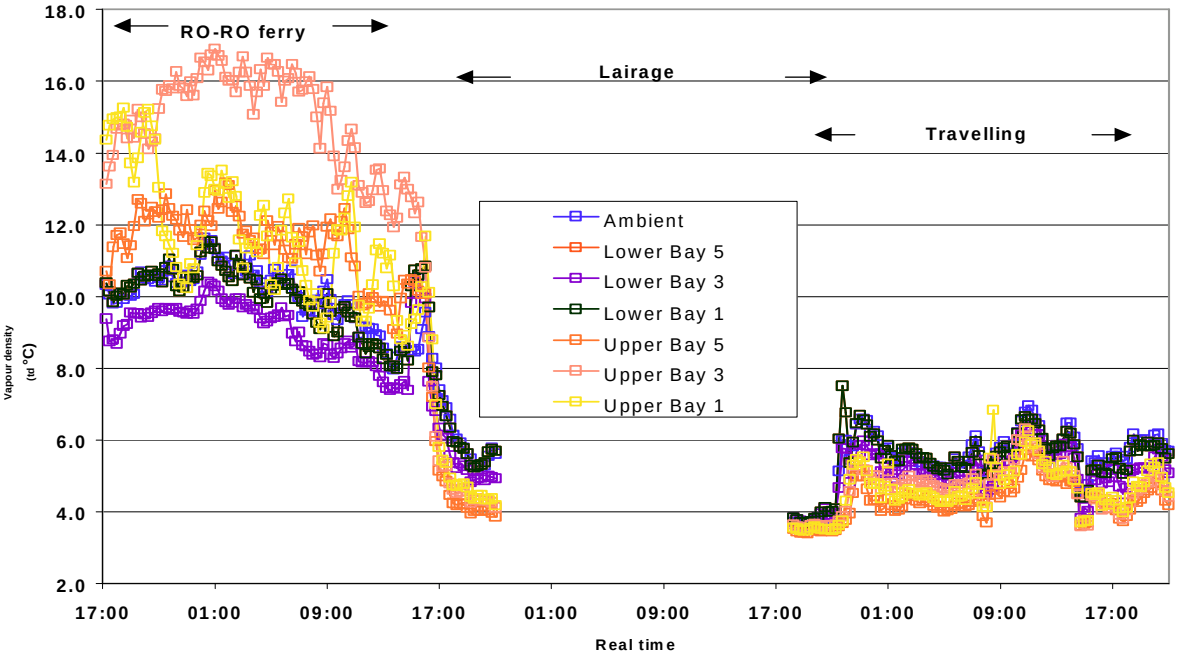


Figure 2: Variation in Vapour Density during transport



7.1.2 Vapour density (td ^{o}C)

The mean ambient vapour density on the RO-RO ferry was 9.4, the mean values for the lower bays of the transporter were 9.3 (Bay 1), 8.5 (Bay 3), 9.5 (Bay 5), and on the upper bays were 10.6 (Bay 1), 13.1 (Bay 3) and 10.2 (Bay 5), respectively. The mean ambient vapour density on the journey from Fougeres to the feedlot in Spain was 5.5 and the mean values for the lower bays were 5.5 (Bay 1), 5.1 (Bay 3), 4.6 (Bay 5), and the upper bays were 4.6 (Bay 1), 4.7 (Bay 3) and 4.3(Bay 5), respectively.

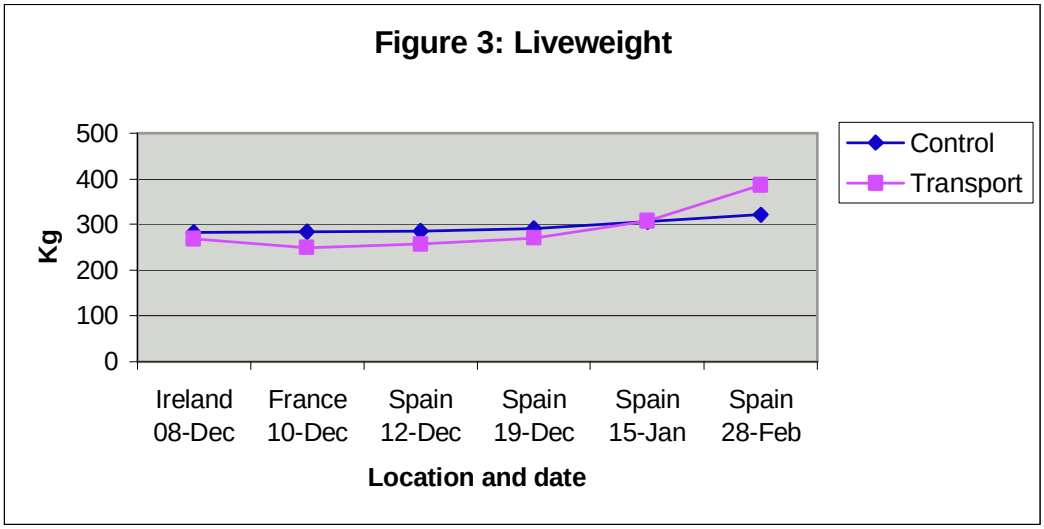
7.2 Liveweight

The changes in liveweight are shown in Tables 2 and Figure 3. There were no differences between the control and transported groups before the journey. There was no significant difference in the rate of gain for either the control or transported animals from day 0 to day 82. The mean liveweight of the transported group decreased by 7.6% by the time of arrival in Fougeres (Day 2). The weight loss following the transport journey may be due to decreased gut fill. Bodyweight increased by 3.3% by time of arrival in the feedlot in Spain. By Day 11, the liveweight of the transported animals was similar to Day 0 values.

The mean daily liveweight gain (kg/day ± sem) for the control and transported treatments were 0.61±0.07 versus 1.03±0.05 from December 8th to January 15th; 0.53±0.06 versus 1.35± 0.04 from December 18th to January 15th; 0.36±0.06 versus 1.74±0.04 from January 15th to February 28th, respectively.

Table 2: Changes in liveweight in control and transported animals. Values are expressed as mean (kg) ± SEM with P values.

Treatment	Statistics	Day 0	Day 3	Day 5	Day 11	Day 38	Day 82
Control	**Mean**	**283**	**284**	**285**	**291**	**306**	**322**
	SEM	8.96	8.39	8.4	8.77	8.98	9.03
	N	28	28	27	28	28	28
Transport	**Mean**	**269**	**249**	**257**	**270**	**308**	**385**
	SEM	6.33	5.93	5.93	6.2	6.35	6.39
	N	52	52	52	52	52	52
	Significance P =	**0.2245**	**0.0009**	**0.0077**	**0.062**	**0.8203**	**0.0001**



7.3 Rectal temperature

Prior to transport (Day 0) the median rectal body temperature for control animals was 38.8°C and ranged from 38 to 41.1°C. The temperatures of the animals assigned to transport were 39°C and ranged from 37.9 to 40.7°C, respectively ($P<0.04$ versus control). In general, the rectal body temperature of transported animals was significantly lower at all time points compared with control animals, with a significant treatment x time interaction ($P < 0.0001$). While body temperature were significantly lower for the transported animals, they were still within the normal clinical range (37.8 - 38.8°C) (Anderson, 1993). In animals, normal cellular function depends on a relatively constant body temperature, which is the sum of heat production (or conservation) and heat loss. This temperature is regulated by a central mechanism within the hypothalamus in the brain which activates both physiological and behavioural activities.

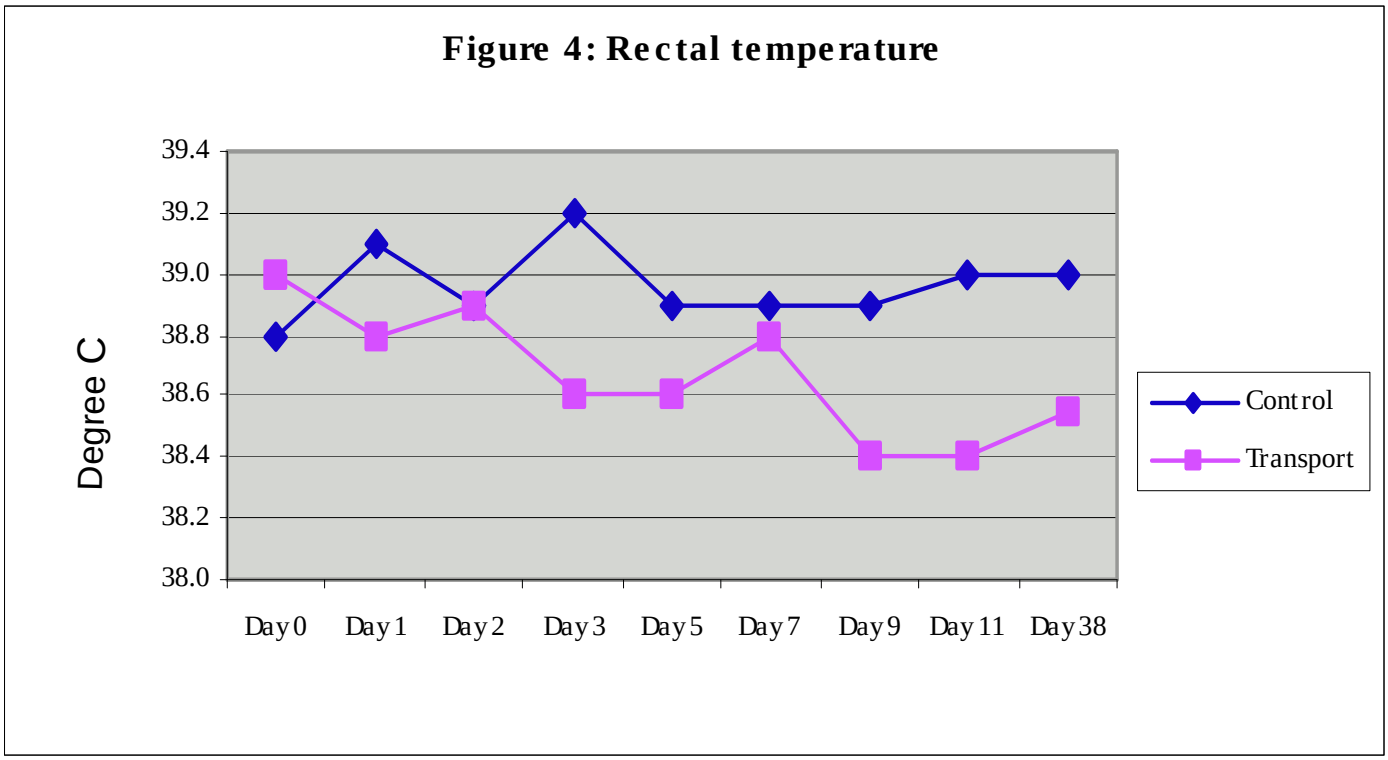
There was no incidence of respiratory disease in the transported animals for the duration of the study. One control animal had an elevated temperature on day 4 and was treated with antibiotics.

Table 3: Changes in rectal temperature (^{o}C) in control and transported animals. ***(The values are expressed as median with minimum and maximum values).***

Treatment	Statistic	Pre-transport Ireland	Arrival in French lairage France	Lairage* France	Lairage** France	Arrival in Spanish Feedlot Spain	Feedlot Spain	Feedlot Spain	Feedlot Spain	Feedlot Spain	Repeated	Sig.
	Day	**0**	**2**	**3**	**3**	**5**	**7**	**9**	**11**	**38**		
Control (C)	**Median**	38.8	39.1	38.9	39.2	38.9	38.9	38.9	39	39	**Treat**	**0.0001**
	Min - Max	38 - 41.1	38.1 - 39.9	38.3 - 39.6	38.6 - 39.7	38.3 - 39.5	38.5 - 40	38.7 - 40	38.4 - 39.8	38.7 - 39.4	**Sample**	**0.2018**
	n	28	28	28	28	28	28	28	28	27	**Treat * Sample**	**0.0001**
	Compare to Pre transport		**P = 0.0417**			**P = 0.4348**			**P = 0.2373**			
Transport (T)	**Median**	39	38.8	38.9	38.6	38.6	38.8	38.4	38.4	38.55		
	Min - Max	37.9 - 40.7	38.2 - 39.8	38.3 - 39.4	37.5 - 40.8	38 - 40.5	37.8 - 39.8	37.5 - 39.9	37.8 - 39.8	38.1 - 39.1		
	n	52	51	52	52	52	52	52	52	52		
	Compare to Pre transport		**P = 0.0083**			**P = 0.0001**			**P = 0.0001**			
	Sig. (C v's T)	**P = 0.0416**	**P = 0.0011**	**P = 0.887**	**P = 0.0001**	**P = 0.0001**	**P = 0.0696**	**P = 0.0001**	**P = 0.0001**	**P = 0.0001**		

***** after 12 hours; ****** after 24 hours;

Rectal temperature in control and transported animals.



7.4 Physiological variables

Table 4: Normal biochemical ranges for cattle, from the Veterinary Laboratories Agency, and normal haematological ranges from Jain (1986) and Radostits and others (1994), Schalm (1961) and Kanenko (1989). Source reference (Knowles et al., 2000).

Biochemistry	Range	Haematology	Range
Haematocrit (%)	24 - 46	Albumin (g/litre)	27 - 39
Haemoglobin (g/dl)	8 - 15	ALP (U/litre)	90 - 170
Lymphocytes (10^9/litre)	2.5 – 7.5	BHB (mmol/litre)	0 - 1.2
MCH (pg)	11 – 17	CK (U/litre)	0 - 200
MCHC (g/dl)	30 – 36	Cortisol *	
Mean Cell Volume (fl)	40 – 60	Creatinine (µmol/litre)	44 - 165
Monocytes (10^9/litre)	0 – 0.8	Iron (µmol/litre)	21 - 41
Neutrophils (10^9/litre)	0.6 – 4.0	Fibrinogen (g/litre)	2 - 5
Platelets (10^9/litre)	100 – 800	Glucose (mmol/litre)	2.8 - 3.6
RBC (10^{12}/litre)	5 – 10	Haptoglobin (g/litre)	0 - 0.04
WBC (10^{12}/litre)	4 – 12	NEFA (µmol/litre)	0 - 600
		Total protein (g/litre)	61 - 81
		Urea (mmol/litre)	3.4 - 7.3

* No estimates available. MCH mean cell haemoglobin; MCHC mean cell haemoglobin concentration**.**

7.4.1 Haematocrit (HCT) %

There was no significant difference for blood haematocrit between treatments prior to transport (Table 5). Following the 23-hour sea journey and 3-hour road journeys to Fougeres (France), blood haematocrit % was decreased 12-hours after arrival (sample 3). There was a significant treatment x sample time interaction with significant differences between treatments at 12 hours after arrival in the french lairage (samples 3), arrival in Spain (sample 5), and at day 9, 11 and 38 of the study (i.e. samples 7, 8 and 9), with transported animals having significant lower HCT percentages. Overall, the HCT % for the transported animals at arrival in France (sample 3) and in Spain (sample 5), and on day 11, were significantly lower than their pre-transport values. While values were decreased, they are within the range of normal referenced ranges (24 - 48%) (Knowles et al., 2000; Schalm, 1961; Schalm, 1984).

7.4.2 Haemoglobin (Hb) and Red blood cell (RBC) numbers

There was no significant difference in blood haemoglobin (Hb) levels prior to transport or 12 hours after arrival in France (Table 6). Blood Hb levels were significantly decreased following arrival in Spain (Sample 5) and remained significantly lower than control values at sampling 7, 8 and 9. Overall, there was a significant treatment x sample interaction ($P < 0.0001$). The Hb concentrations for the transported animals at arrival in France (sample 3) and in Spain (sample 5), and on day 11, were significantly lower than their pre-transport values.

The function of red blood cells (RBC) is to carry oxygen to the tissues at pressures sufficient to permit rapid diffusion of oxygen. Interference with synthesis or release of Hb, production or survival of RBC, or metabolism causes disease.

There was no significant difference in red blood cell numbers prior to transport or at sample 3 (12 hours after arrival in France) (Table 7). There was a significant treatment x sample interaction with lower RBC concentrations at samples 5 (arrival in Spain), 6 (day 7), 7 (Day 9), 8 (Day 11) and 9 (Day 38) and the RBC numbers were significantly lower than their pre-transport values. Under sympathetic-adrenal adrenal stimulation, haematocrit values may be increased by the contraction of the spleen, which release erythrocytes into the circulation, thus reflecting the increase in RBC concentrations. The RBC numbers and haemoglobin were within the normal blood referenced ranges ((RBC; 5.0 – 10.0 $x10^6$ ul) (haemoglobin 8-14 g%)(Schalm, 1961) and Table 4)).

7.4.3 White blood cell (WBC) number

There was no significant difference in WBC numbers prior to transport (Table 8). Following transportation by road, sea and 12 hours after arrival in France, WBC numbers were significantly increased in transported animals (sample 3, day 3 of the study). WBC numbers were significantly decreased compared with control values on arrival in Spain (sample 5) and at sample 6, 7 and 8 with no significant difference in WBC numbers by day 11 (sample 9) There was a significant treatment x sample interaction ($P < 0.001$). The only sampling point at which white blood counts increased above the upper limit of 12, was at the sample collected 12 hours after arrival in the french lairage.

White blood cells (leukocytes) are less than 1% of the blood's volume. They are made from stem cells in bone marrow. There are five types of leukocytes, important components of the immune system. Neutrophils enter the tissue fluid by squeezing through capillary walls and phagocytozing foreign substances. Lymphocytes fight infection. T-cells attack cells containing viruses. B-cells produce antibodies. The normal referenced ranges for total blood leukocytes is 4 – 12 (mean 8.00) (Schalm, 1961).

7.4.4 Lymphocyte and monocyte percentages (%)

There was no significant change in lymphocyte (Table 9) or monocyte (Table 10) numbers pre-transport. Following transport, the % lymphocytes were decreased at sample 5 (arrival in Spain), at samples 6, 7, 8 and 9 when compared with control values. The % monocytes were significantly increased at sample 5 (arrival in Spain) and decreased at sample 6, 7, 8, and 9. There was a significant treatment x sample interaction for samples 1 to 9 (Day 0 to day 38 of the study). Lymphocytes are responsible for both humoral and cellular immunity. Cells of the two branches of the immune system cannot be differentiated morphologically, but they differ in their dynamics of production and circulation.

7.4.5 Neutrophil percentage (%)

There was no significant difference in the percentages of blood neutrophils prior to transport or in the 12 hour period after arrival in France (Table 11). There was a significant treatment x sample interaction at samples 5 (arrival in Spain), 6 (Day 7), 7 (Day 9), 8 (Day 11) and 9 (Day 38) with values remaining significantly higher compared with control animals. Changes in the populations of white blood cell types (leukocytes, monocytes) in response to stressors, particularly the relative decrease in lymphocyte compared with neutrophil numbers have been measured in studies relevant to animal welfare. The normal referenced ranges for differential counts, neutrophils are in the range 15-45 (Schalm, 1961; Table 4)).

Table 5: Haematocrit % in control and transported animals (samples 1 to 9). Pre-transport measurements were taken at –24 hours (day 0). ***Values are expressed as Mean ± SD*** **with P values.**

			Pre-transport	Arrival in French lairage	Arrival in Spanish Feedlot	Feedlot	Feedlot	Feedlot	Feedlot		
			Ireland	France *	Spain	Spain	Spain	Spain	Spain		
		Day	0	2	5	7	9	11	38		
Variable	Treatment	Statistic	Sample 1	Sample 3	Sample 5	Sample 6	Sample 7	Sample 8	Sample 9	Repeated	Sig. P =
Haematocrit %	Control (C)	Mean	39.2	39.3	38	38.4	38.2	37	38.6	Treat	0.0001
		SD	4.61	3.93	3.86	3.87	3.77	3.52	4.15	Sample	0.0001
		N	27	28	28	28	28	28	27	Treat * Sample	0.0001
		Compare to Sample 1		P = 0.8698	P = 0.0260			P = 0.0004			
	Transport (T)	Mean	38.8	36.7	33.2	37.1	32.8	31.6	35.6		
		SD	3.54	2.96	2.81	3.64	5	2.56	4.42		
		N	51	52	51	52	52	52	52		
		Compare to Sample 1		P = 0.0001	P = 0.0001			P = 0.0001			
		Sig. (C v's T)	P = 0.665	P = 0.0032	P = 0.0001	P = 0.128	P = 0.0001	P = 0.0001	P = 0.01		

Table 6: Plasma haemoglobin concentrations in control and transported animals (samples 1 to 9). Pre-transport measurements were taken at –24 hours (day 0). ***Values are expressed as Mean ± SD*** **with P values.**

Haemoglobin											
Hb	Treatment	Statistic	Sample 1	Sample 3	Sample 5	Sample 6	Sample 7	Sample 8	Sample 9	Repeated	Sig. P =
g%	Control (C)	Mean	13.7	13.6	13.2	13.4	13.3	12.8	13.4	Treat	0.0001
		SD	1.58	1.34	1.28	1.32	1.32	1.23	1.49	Sample	0.0001
		N	27	28	28	28	28	28	27	Treat * Sample	0.0001
		Compare to sample 1		P = 0.3939	P = 0.0037			P = 0.0001			
	Transport (T)	Mean	13.5	13.3	11.1	12.8	11.3	10.9	11.9		
		SD	1.17	1.11	0.8	1.36	0.97	0.69	1.26		
		N	51	52	51	52	52	52	52		
		Compare to sample 1		P = 0.1316	P = 0.0001			P = 0.0001			
*after 12 hours		Sig. (C v's T)	P = 0.4145	P = 0.3322	P = 0.0001	P = 0.0769	P = 0.0001	P = 0.0001	P = 0.0001		

Table 7: Red Blood Cell (RBC) numbers in control and transported animals (samples 1 to 9). Pre-transport measurements were taken at –24 hours (day 0). *Values are expressed as Mean ± SD* with P values.

			Pre-transport	Arrival in French lairage	Arrival in Spanish Feedlot	Feedlot	Feedlot	Feedlot	Feedlot		
			Ireland	France*	Spain	Spain	Spain	Spain	Spain		
Red blood cells		**Day**	**0**	**2**	**5**	**7**	**9**	**11**	**38**		
RBC	**Treatment**	**Statistic**	**Sample 1**	**Sample 3**	**Sample 5**	**Sample 6**	**Sample 7**	**Sample 8**	**Sample 9**	**Repeated**	**Sig. P =**
1 X 10^6 µl	**Control ©**	**Mean**	11.15	11.12	10.79	10.93	10.79	10.44	10.53	**Treat**	**0.0001**
		SD	1.697	1.355	1.342	1.434	1.366	1.311	1.252	**Sample**	**0.0001**
		N	27	28	28	28	28	28	27	**Treat * Sample**	**0.0001**
		Compare to sample 1		**P = 0.5197**	**P = 0.0506**			**P = 0.0008**			
	Transport (T)	**Mean**	11.38	10.68	7.88	9.06	8.07	7.54	8.59		
		SD	1.206	0.981	0.74	0.961	0.883	0.741	0.989		
		N	51	52	51	52	52	52	52		
		Compare to sample 1		**P = 0.0001**	**P = 0.0001**			**P = 0.0001**			
		Sig. (C v's T)	**P = 0.4716**	**P = 0.2682**	**P = 0.0001**	**P = 0.0001**	**P = 0.0001**	**P = 0.0001**	**P = 0.0001**		
Normal Range	1x10^{12}/l	(Knowles et al., 2000)									

Table 8: White blood Cell (WBC) numbers in control and transported animals (samples 1 to 9). Pre-transport measurements were taken at –24 hours (day 0). *Values are expressed as Mean ± SD* with P values.

White blood cells (WBC)	**Treatment**	**Statistic**	**Sample 1**	**Sample 3**	**Sample 5**	**Sample 6**	**Sample 7**	**Sample 8**	**Sample 9**	**Repeated**	**Sig. P =**
1 X 10^3 µl	**Control ©**	**Median**	11.2	10.45	8.8	8.95	9.2	8.95	9.1	**Treat**	**0.0403**
		Min – Max	7.3- 16.9	7.4 – 16.4	6.5 – 14.9	5.8 – 15.3	6.4 – 14.1	6.2 – 13.7	6.3 – 12.2	**Sample**	**0.1436**
		N	27	28	28	28	28	28	27	**Treat * Sample**	**0.0001**
		Compare to sample 1		**P = 0.1425**	**P = 0.0001**			**P = 0.0001**			
	Transport (T)	**Median**	10.8	14	8.22	7.345	7.06	7.545	7.91		
		Min – Max	5.4 –20.1	7.5 – 24	4.04 – 14.01	4.62 – 15.46	4.08 – 14.71	4.1 – 15.74	5.06 – 13.53		
		N	51	52	51	52	52	52	52		
		Compare to sample 1		**P = 0.0001**	**P = 0.0001**			**P = 0.0001**			
*after 12 hours		**Sig. (C v's T)**	**P = 0.5139**	**P = 0.0001**	**P = 0.0 296**	**P = 0.0014**	**P = 0.0001**	**P = 0.0159**	**P = 0.1123**		

Table 9: Lymphocyte % in control and transported animals (samples 1 to 9). Pre-transport measurements were taken at –24 hours (day 0). *Values are expressed as Median with minimum and Maximum values* with P values.

			Pre-transport	Arrival in French lairage	Arrival in Spanish Feedlot	Feedlot	Feedlot	Feedlot	Feedlot		
			Ireland	France *	Spain	Spain	Spain	Spain	Spain		
		Day	0	2	5	7	9	11	38		
Lymphocyte %	Treatment	Statistic	Sample 1	Sample 3	Sample 5	Sample 6	Sample 7	Sample 8	Sample 9	Repeated	Sig. P =
	Control (C)	Median	64	60.5	69.5	69.5	73	72	70	Treat	0.0001
		Min - Max	37- 75	41 - 79	43 - 83	45 - 84	42 - 82	56 - 85	52 - 85	Sample	0.0679
		N	27	28	28	28	28	28	27	Treat * Sample	0.0001
		Compare to sample 1		P = 0.1370	P = 0.2407			P = 0.0001			
	Transport (T)	Median	64	62	32	38	38	40	40		
		Min - Max	39 - 79	15 - 81	25 - 71	24 - 47	26 - 50	24 - 86	32 - 51		
		N	51	52	51	52	52	52	52		
		Compare to sample 1		P = 0.0882	P = 0.0001			P = 0.0001			
		Sig. (C v's T)	P = 0.9088	P = 0.9095	P = 0.0001	P = 0.0001	P = 0.0001	P = 0.0001	P = 0.0001		
Normal range	45-75	(58)	Schalm, 1961								

Table 10: Monocyte % in control and transported animals (samples 1 to 9). Pre-transport measurements were taken at –24 hours (day 0). *Values are expressed as Median with minimum and Maximum values* with P values.

Monocyte %	Treatment	Statistic	Sample 1	Sample 3	Sample 5	Sample 6	Sample 7	Sample 8	Sample 9	Repeated	Sig. P =
	Control (C)	Median	2	3	3	3	3	3	3	Treat	0.0001
		Min – Max	0 - 6	0 - 7	1 - 6	1 - 5	1 - 7	1 - 5	1 - 5	Sample	0.023
		N	27	28	28	28	28	28	27	Treat * Sample	0.0001
		Compare to sample 1		P = 0.3314	P = 0.1660			P = 0.3497			
	Transport (T)	Median	3	6	2	2	2	2	1		
		Min – Max	1 - 8	1 - 22	1 - 4	1 - 3	1 - 3	1 - 4	1 - 3		
		N	51	52	51	52	52	52	52		
		Compare to sample 1		P = 0.0001	P = 0.0004			P = 0.0001			
		Sig. (C v's T)	P = 0.2506	P = 0.0001	P = 0.0007	P = 0.0001	P = 0.0001	P = 0.0001	P = 0.0001		
*after 12 hours											

Table 11: Neutrophil % in control and transported animals (samples 1 to 9). Pre-transport measurements were taken at –24 hours (day 0). *Values are expressed as Median with minimum and Maximum values* with P values.

			Pre-transport	Arrival in French lairage	Arrival in Spanish Feedlot	Feedlot	Feedlot	Feedlot	Feedlot			
			Ireland	France*	Spain	Spain	Spain	Spain	Spain			
		Day	0	2	5	7	9	11	38			
Neutrophil %	Treatment	Statistic	Sample 1	Sample 3	Sample 5	Sample 6	Sample 7	Sample 8	Sample 9	Repeated	Sig. P =	
	Control ©	Median	30	33.5	23	23	22	24.5	26	Treat		0.0001
		Min – Max	17 – 52	16 – 56	13 – 55	11 – 52	12 – 51	13 – 40	10 – 45	Sample		0.0614
		N	27	28	28	28	28	28	27	Treat * Sample		0.0001
		Compare to sample 1		P = 0.2545	P = 0.1077			P = 0.0017				
	Transport (T)	Median	28	28	62	58.5	59	56	55			
		Min – Max	12 – 49	10 – 68	25 – 72	47 – 72	47 – 70	36 – 69	42 – 63			
		N	51	52	51	52	52	52	52			
		Compare to sample 1		P = 0.6614	P = 0.0001			P = 0.0001				
		Sig. (C v's T)	P = 0.8629	P = 0.144	P = 0.0001	P = 0.0001	P = 0.0001	P = 0.0001	P = 0.0001			

*After 12 hours

7.4.6 Albumin

There was no significant difference in blood albumin concentrations prior to transport (Table 12). There was a significant increase in albumin concentrations following the transport journey to France, and concentrations remained elevated at 12 and 24 hours after arrival. There was a significant treatment by sample interaction. Albumin concentrations returned to control levels by sample 9 (day 38 of the study).

Serum albumin measurements are used in the diagnosis and treatment of numerous diseases involving primarily the liver and kidney. Albumin has two major functions within the body. Albumin creates an osmotic gradient between the inside of blood vessels and the surrounding tissues. Without this, water migrates into the tissues and oedema develops. Normal levels of Albumin in the blood prevent this from happening. The only cause of increased albumin is dehydration; there is no naturally occurring hyperalbuminemia. Dehydration leads to hemoconcentration through reduction in fluid volume and consequently hyperprotienaemia.

7.4.7 Aspartate amino transferase (AST)

There was no significant difference between treatments in AST concentrations prior to transport, but levels were significantly increased 24 hours after arrival in France, on arrival in Spain and at sample 5 (Day 5), 6 (Day 7), 7 (Day 9), 8 (Day 11), and 9 (Day 38) (Table 13; Figure 13). There was a significant treatment by sample interaction.

Values were significantly raised in all treatments (control and transport) at sample 2 (time 0 arrival in France) when compared with pre-transport concentrations. However, values in transported animals were still within normal physiological blood levels. The AST concentrations for the transported animals at arrival in France and Spain were not significantly different from their pre-transport concentrations but were increased at day 11 (sample 8) relative to pre-transport values.

AST catalyses the transamination of L-aspartate and 2-oxoglutarate to oxaloacetate and glutamate. The presence of AST in so many tissues makes their serum level a good marker of soft tissue damage but precludes its use as an organ-specific enzyme.

7.4.8 Interferon-gamma production (IFN-γ)

The stimulated production of interferon-γ in response to concanavalin-A (Con-A) showed a significant treatment by sample interaction with transported animals having significantly lower levels than controls at sample 3 (12 hours after arrival in France) (with levels returning to control values 24 hours after arrival in France) (Table 14a). IFN-γ levels were significantly lower at samples 5 (after journey through France to Spain), 6 (Day 7), 7 (Day 9) and 8 (Day 11) with values returning to control levels by sample 9 (Day 38). When comparing the CON-A induced interferon-γ levels for the transported animals with their pre-transport levels, there was a significant decrease at sample 5 (day of arrival in Spain) and at sample 8 (Day 11 of the study).

The stimulated production of interferon-γ in response to the mitogen keyhole limpet haemocyanin (KLH) was significantly lower than control animals at sample 2 (day of arrival in France), 3, (12 hours post-arrival), sample 4 (24 hours post-arrival), sample 5 (arrival in Spain) and at samples 6 (Day 7), 7 (Day 9) and 8 (Day 11) (Table 14b). When comparing the KLH- induced interferon-γ levels for the transported animals with their pre-transport levels, there was a significant decrease at sample 2 (day of arrival in France) with no significant difference at sample 5 (day of arrival in Spain) or 8 (Day 11 of the study). Interferon-γ is produced by activated T lymphocytes and natural killer (NK) cells in response to antigen.

The mitogen (keyhole limpet haemocyanin KLH) - and antigen (Concanavalin-A (Con-A))-induced in vitro interferon-γ production was used as an indicator of cell-mediated immunity and is a useful and sensitive indicator of changes in immune function. As the technique does not necessarily

require repeated differential centrifugation to isolate lymphocytes, it is also more practical from a methodological aspect than measurements of lymphocyte blastogenesis, with larger numbers of blood samples able to be handled at one time.

7.4.9 Creatine kinase (CK)

There was a significant increase in CK activities at samples 2, (arrival in France), 3, (12 hour post-arrival in France), 4 (24 hours post-arrival in France), at sample 5 (after arrival in Spain), and at samples 6, 7 and 8 when compared with control activities (Table 15). CK activities returned to control levels by day 38 of the study (Sample 9). When the values for the transported animals were compared with their pre-transport baseline values, activities were significantly higher at sample 2, 5 and 8. It is also important to indicate that the CK activites of the transported animals while significantly higher than control values are still within the normal physiological range.

CK iso-enzymes are the most organ specific serum enzymes in clinical use. They catalyse the reversible phosphorylation of creatine to ATP to form creatine phosphate, the major storage form of high-energy phosphate required by muscle.

7.5.0 Glucose

There was a significant increase in glucose concentrations at samples 2, (time 0 arrival in France), 3, (12 hours post-arrival in France), 4 (24 hours post-arrival in France), at sample 5 (arrival in Spain), and at samples 6 and 8 when compared with control values (Table 16). When the values for the transported animals were compared with their pre-transport baseline values, glucose concentrations were significantly higher at sample 2 (day of arrival in France). Carbohydrate in the form of glucose is the principal source of energy for the body.

7.5.1 Acute phase proteins (Haptoglobin and Fibrinogen)

Pre-transport plasma haptoglobin and fibrinogen concentrations were not significantly different indicating that there were no underlying inflammatory conditions existing in the animals (Table 17). Plasma haptoglobin values were significantly increased in the transported animals at sample 2, 3, 5, 6, 7, and 9 when values were compared with control animals. Transported animals had significantly higher levels at arrival in France (Sample 2) and Spain (Sample 5) and at sample 8 (day 11 of the study) when compared with pre-transport baseline concentrations.

Plasma fibrinogen levels were significantly increased in the transported animals at sample 2 (time 0 arrival in France) when values were compared with control animals (Table 18). Transported animals had significantly higher levels at arrival in France (sample 2) when compared with their pre-transport baseline fibrinogen concentrations (Day 0).

Inflammation resulting from stress can cause the release of acute phase proteins (APP) such as haptoglobin and fibrinogen, and APP in cattle have been associated with immunosuppression and much higher levels have bee reported in inflammatory conditions (Earley et al., 2002).

7.5.2 Lactate

Pre-transport plasma lactate concentrations were not significantly different (Table 19). Following transportation, animals had significantly higher lactate concentrations at sample 2 (arrival in France), 5 (arrival in Spain), 6 (Day 7), 7 (Day 9) and 8 (Day 11) when compared with control values. Transported animals had significantly higher values at arrival in Spain (Sample 5; Day 5) and at sample 8 (Day 11) when compared with their pre-transport baseline lactate concentrations.

7.5.3 Non-esterified fatty acids (NEFA)

Pre-transport NEFA concentrations were not significantly different. Following transport, transported animals had significantly higher NEFA concentrations at all blood sampling times

(Samples 2 to 9) (Table 20) compared with the controls. Transported animals had significantly higher levels at arrival in France (Sample 2; Day 2) and Spain (Sample 5; Day 5) and at sample 8 (Day 11) when compared with their pre-transport baseline NEFA concentrations. Control animals had significantly higher levels at sample 5 (Day 5) when compared with their pre-transport baseline NEFA concentrations. However, levels are within the normal referenced ranges (Knowles et al., 2000).

7.5.4 Urea

Pre-transport urea concentrations were not significantly different. Following transport, transported animals had significantly higher urea concentrations at blood sampling times 2, 3, 4 and 9 when compared with control values (Table 21). Transported animals had significantly higher levels at arrival in France (Sample 2; Day 2) and lower levels at sample 5 (Day of arrival in Spain) and at sample 8 (Day 11) when compared with their pre-transport baseline urea concentrations.

7.5.5 Beta-hydroxybutyrate (BHB)

Pre-transport BHB concentrations were not significantly different (Table 22). Following transportation animals had significantly lower BHB concentrations at sampling times 2 and 4 with significantly higher concentrations at sample 5 and 6 and lower levels at sample 8 and 9 when compared with the controls. They had significantly lower levels at arrival in France (Sample 2; Day 2) and higher levels at sample 5 (Day of arrival in Spain) and lower levels at sample 8 (Day 11) when compared with their pre-transport baseline BHB concentrations.

7.5.6 Protein

There was no significant difference prior to transport in protein concentrations (Table 23). Following transport, a significant increase in plasma protein concentration was measured at samples 2 to 9 inclusive. This increase was within the normal referenced ranges for plasma protein (67.4-74.6g/l, Reference; Kaneko, 1989). Transported animals had significantly higher levels at arrival in France (Sample 2; Day 2) and at sample 5 (Day of arrival in Spain) when compared with their pre-transport baseline protein concentrations and control animals had significantly lower protein concentrations at sample 2, 5 and 8 when compared with their baseline values on Day 0. Protein measurement along with Albumin can indicate whether there has been an antibody response. (Total Protein - Albumin = Globulin) an increase in Gamma Globulins and a series of Acute Phase Proteins can result in an increase in the total Protein but this is usually somewhat offset by the reduction of Albumin in all Acute Phase situations. (Albumin is a "Reverse" Acute Phase Protein). Total Protein by itself can in no way diagnose liver damage or disease.

7.5.7 Lactate dehydrogenase (LDH)

There was no significant difference prior to transport in LDH activity (Table 24). Following transport, a significant increase in plasma LDH activities was measured at samples 2, 6, 7 and 8. Transported animals had significantly higher values at arrival in France (Sample 2; Day 2) and at sample 8 (Day 11) when compared with their pre-transport baseline LDH values and control animals had significantly lower LDH levels sample 2, 5 and 8 when compared with their baseline values on Day 0. This is also an intracellular non-specific enzyme found in numerous tissues along with kidney, heart, skeletal muscle, brain, liver and lungs. Increases are usually found in cellular death and/or leakage from the cell. Decreased levels of the enzyme may be seen in cases of malnutrition, hypoglycemia, adrenal exhaustion or low tissue or organ activity.

7.5.8 Cortisol

There was no significant change in plasma cortisol concentrations in transported animals at arrival in France (Sample 2; Day 2) or at sample 5 (arrival in Spain). On Day 11, the plasma cortisol concentrations of transported animals were significantly higher than control animals (Table 25). Control and transported animals had significantly higher cortisol values at sample 2 (Day 2) compared with their baseline cortisol concentrations (Day 0).

Stressful situations such as exercise or transportation activate the hypothalamic-pituitary-adrenal axis resulting in an increase in plasma cortisol. Cortisol release due to stress may lead to neutrophilia and lymphopenia and increase the neutrophil:lymphocyte ratio. One could tentatively conclude that a mean value of >70 ng/ml in either steers or cows would possibly be an indicator of either rough handling or poor equipment, and low values close to the baseline values would indicate that a procedure was either low stress or was very quick. It must be remembered that cortisol is a time-dependent measure that takes 10 to 20 min to reach peak values. In the present study, plasma cortisol levels in transported animals were significantly lower at sample 3 (12 hours after arrival in France) and were significantly higher on day 11 of the study compared with control values.

Cortisol is a useful indicator of short-term stresses from handling or husbandry procedures such as castration (Earley et al., 2002). Mean plasma cortisol concentrations are generally less than 10 ng/ml for control 6-month old calves that are habituated to blood sampling (Earley et al., 2002), while following castration, cortisol concentrations of castrated calves increased rapidly, reaching an initial peak of 43.3 ± 5.9 ng/ ml within 15 minutes of the procedure. It is of interest to note that on Day 38 of the study cortisol concentrations were significantly higher in both control and transported animals relative to their baseline measurement on day 0. This would suggest that the handling procedure with restraint possibly contributed to the raised cortisol concentrations.

Table 12: Plasma albumin concentrations in control and transported animals (samples 1 to 9). Pre-transport measurements were taken at –24 hours (day 0). *Values are expressed as Median with minimum and Maximum values* with P values.

			Pre-transport	Arrival in French lairage	Lairage *	Lairage ** Depart for Spain	Spanish Feedlot	Feedlot	Feedlot	Feedlot	Feedlot		
		Day	0	2	3	3	5	7	9	11	38		
	Non Parametric Data - Repeated Measures Analysis of Variance												
Variable	**Treatment**	**Statistic**	**Sample 1**	**Sample 2**	**Sample 3**	**Sample 4**	**Sample 5**	**Sample 6**	**Sample 7**	**Sample 8**	**Sample 9**	**Repeated**	**Sig. P =**
ALBUMIN g/l	**Control**	**Median**	37.75	36.95	36.1	36.5	36.5	35.55	35.8	34.8	35.9	**Treat**	**0.0078**
		Min – Max	32.2 - 40.7	32.6 - 40.4	32 - 40.1	32.6 - 40.1	32.3 - 40.3	31.7 - 39.8	32.2 - 39.0	31.4 - 39.0	32.7 - 40.1	**Sample**	**0.3555**
		N	28	28	27	28	28	28	28	28	27	**Treat * Sample**	**0.0001**
		Compare to Sample 1		**P = 0.0189**			**P = 0.0459**			**P = 0.0001**			
	Transport (T)	**Median**	37.35	39.2	37.45	38.5	37.75	37.45	37.05	36.95	36.85		
		Min - Max	30.1 - 40.8	33.9 - 42.1	32.8 - 41.8	35.5 - 44.7	32.6 - 41.3	30.6 - 44.3	30.3 - 40.9	31.7 - 40.4	32.2 -40.3		
		N	52	52	52	52	52	52	48	52	52		
		Compare to Sample 1		**P = 0.0001**			**P = 0.0127**			**P = 0.3105**			
		Sig. (C v's T)	**P = 0.4533**	**P = 0.0001**	**P = 0.0238**	**P = 0.0001**	**P = 0.0743**	**P = 0.0181**	**P = 0.0622**	**P = 0.0017**	**P = 0.6242**		
Normal range	30.3 – 35.5 g/l	(32.9 ± 1.3)	(Kaneko, 1989)										

Table 13: Plasma aspartate aminotransferase (AST) concentrations in control and transported animals (samples 1 to 9). Pre-transport measurements were taken at –24 hours (day 0). *Values are expressed as Median with minimum and Maximum values* with P values.

Variable	**Treatment**	**Statistic**	**Sample 1**	**Sample 2**	**Sample 3**	**Sample 4**	**Sample 5**	**Sample 6**	**Sample 7**	**Sample 8**	**Sample 9**	**Repeated**	**Sig. P =**
AST	**Control (C)**	**Median**	68	60.5	59	63	53.5	54	53.5	50	64	**Treat**	**0.0001**
U/l		**Min - Max**	43 - 123	44 - 110	47 - 92	46 - 90	33 - 90	38 - 117	37 - 90	39 - 76	44 - 154	**Sample**	**0.3439**
		N	28	28	27	28	28	28	28	28	27	**Treat * Sample**	**0.0001**
		Compare to Sample 1		**P = 0.0045**			**P = 0.0001**			**P = 0.0002**			
	Transport (T)	**Median**	65.5	64	65.5	70	65	90	83.5	75	73		
		Min - Max	42 - 143	46 - 142	41 - 193	47 - 207	39 - 142	49 - 207	51 - 156	50 - 178	26 - 126		
		N	52	52	52	52	52	51	48	52	52		
		Compare to Sample 1		**P = 0.6962**			**P = 0.8789**			**P = 0.0271**			
		Sig. (C v's T)	**P = 0.9995**	**P = 0.128**	**P = 0.5574**	**P = 0.0202**	**P = 0.0132**	**P = 0.0001**	**P = 0.0001**	**P = 0.0001**	**P = 0.0424**		
Normal range	78 – 132 U/l	(105 ± 27)	(Kaneko, 1989)										

* after 12 hours; ** after 24 hours

Table 14a: Interferon-γ production in response to Concanavalin-A (Con-A) in control and transported animals (samples 1 to 9). Pre-transport measurements were taken at –24 hours (day 0). *Values are expressed as Median with minimum and Maximum values* with P values.

			Pre-transport	Arrival in French lairage	Lairage *	Lairage ** Depart for Spain	Spanish Feedlot	Feedlot	Feedlot	Feedlot	Feedlot		
		Day	0	2	3	3	5	7	9	11	38		
Variable	**Treatment**	**Statistic**	**Sample 1**	**Sample 2**	**Sample 3**	**Sample 4**	**Sample 5**	**Sample 6**	**Sample 7**	**Sample 8**	**Sample 9**	**Repeated**	**Sig. P =**
CON-A	**Control (C)**	**Median**	0.167	0.254	0.263	0.3165	0.2555	0.2195	0.2745	0.265	0.223	**Treat**	**0.002**
Interferon		**Min - Max**	0.028 - 0.956	0.0171 - 1.492	0.004 - 2.023	0.034 - 1.742	0.039 - 1.54	0 - 1.297	0.06 - 1.572	0.029 - 1.351	-0.033 - 2.244	**Sample**	**0.8457**
γ		**N**	28	28	28	28	28	28	28	28	27	**Treat * Sample**	**0.0001**
		Compare to Sample 1		**P = 0.7383**			**P = 0.0735**			**P = 0.0676**			
	Transport (T)	**Median**	0.1915	0.1895	0.121	0.1805	0.089	0.0665	0.0835	0.1165	0.152		
		Min - Max	0.014 - 1.904	0.001 - 1.343	0.024 - 1.044	0.005 - 0.842	-0.002 - 0.56	-0.006 - 1.113	-0.028 - 0.661	-0.043 - 0.868	-0.008 - 1.0		
		N	52	52	52	52	52	52	52	52	52		
		Compare to Sample 1		**P = 0.1299**			**P = 0.0004**			**P = 0.0081**			
		Sig. (C v's T)	**P = 0.4203**	**P = 0.5634**	**P = 0.0029**	**P = 0.0801**	**P = 0.0001**	**P = 0.0025**	**P = 0.0001**	**P = 0.0141**	**P = 0.3043**		

Table 14b: Interferon-γ production in response to Keyhole limpet haemocyanin (KLH) in control and transported animals (samples 1 to 9). Pre-transport measurements were taken at –24 hours (day 0). *Values are expressed as Median with minimum and Maximum values* with P values.

Variable	**Treatment**	**Statistic**	**Sample 1**	**Sample 2**	**Sample 3**	**Sample 4**	**Sample 5**	**Sample 6**	**Sample 7**	**Sample 8**	**Sample 9**	**Repeated**	**Sig. P =**
KLH	**Control (C)**	**Median**	0.0115	0.022	0.029	0.0335	0.043	0.0415	0.0675	0.069	0.042	**Treat**	**0.0001**
Interferon γ		**Min - Max**	-0.055 - 0.357	-0.011 - 0.192	-0.005 - 0.358	-0.022 - 0.647	-0.026 - 0.895	-0.016 - 0.456	-0.019 - 0.684	-0.031 - 1.736	-0.063 - 0.168	**Sample**	**0.9582**
		N	28	28	28	28	28	28	28	28	27	**Treat * Sample**	**0.0041**
		Compare to Sample 1		**P = 0.9908**			**P = 0.1530**			**P = 0.0251**			
	Transport (T)	**Median**	0.0155	0.007	0.004	0.013	0.0025	0.006	0.0205	0.025	0.02		
		Min - Max	-0.009 - 0.2	-0.096 - 0.07	-0.014 - 0.256	-0.012 - 0.177	-0.072 - 0.458	-0.084 - 0.194	-0.084 - 0.431	-0.067 - 0.336	-0.054 - 0.563		
		N	52	52	52	52	52	52	52	52	52		
		Compare to Sample 1		**P = 0.0102**			**P = 0.4458**			**P = 0.7017**			
		Sig. (C v's T)	**P = 0.6391**	**P = 0.0007**	**P = 0.0001**	**P = 0.0027**	**P = 0.0002**	**P = 0.0002**	**P = 0.0006**	**P = 0.0005**	**P = 0.0942**		

* after 12 hours; ** after 24 hours

Table 15: Plasma creatine kinase (CK) concentrations in control and transported animals (samples 1 to 9). Pre-transport measurements were taken at –24 hours (day 0). ***Values are expressed as Median with minimum and Maximum values*** **with P values.**

			Pre-transport	Arrival in French lairage	Lairage *	Lairage ** Depart for Spain	Spanish Feedlot	Feedlot	Feedlot	Feedlot	Feedlot		
		Day	0	2	3	3	5	7	9	11	38		
Variable	**Treatment**	**Statistic**	**Sample 1**	**Sample 2**	**Sample 3**	**Sample 4**	**Sample 5**	**Sample 6**	**Sample 7**	**Sample 8**	**Sample 9**	**Repeated**	**Sig.**
Creatine kinase	**Control (C)**	**Median**	184.5	167	227	252.5	152	129	131.5	120.5	161	**Treat**	**0.0001**
µmol/l		**Min - Max**	91 - 1581	86 - 594	120 - 2341	118 - 1521	92 - 1026	86 - 445	85 – 244	72 - 197	95 - 452	**Sample**	**0.4302**
		N	28	28	27	28	28	28	28	28	27	**Treat * Sample**	**0.0001**
		Compare to Sample 1		**P = 0.3400**			**P = 0.4365**			**P = 0.0271**			
	Transport (T)	**Median**	176	**257**	**403.5**	**422**	**266.5**	**1253**	**338.5**	156	159.5		
		Min - Max	106 - 794	116 - 4115	140 - 5078	141 - 4080	92 - 3250	415 - 3054	171 – 670	86 - 361	80 - 736		
		N	52	52	52	52	52	51	48	52	52		
		Compare to Sample 1		**P = 0.0013**			**P = 0.0041**			**P = 0.0415**			
		Sig. (C v's T)	**P = 0.7556**	**P = 0.0002**	**P = 0.0011**	**P = 0.0002**	**P = 0.0001**	**P = 0.0001**	**P = 0.0001**	**P = 0.0001**	**P = 0.5494**		
Normal range	88.4 - 177 µmol/l	Kaneko, 1989											

Table 16: Plasma glucose concentrations in control and transported animals (samples 1 to 9). Pre-transport measurements were taken at –24 hours (day 0). ***Values are expressed as Median with minimum and Maximum values*** **with P values.**

Variable	**Treatment**	**Statistic**	**Sample 1**	**Sample 2**	**Sample 3**	**Sample 4**	**Sample 5**	**Sample 6**	**Sample 7**	**Sample 8**	**Sample 9**	**Repeated**	**Sig. P =**
GLUCOSE	**Control (C)**	**Median**	4.4	4.3	4.55	4.45	4.5	4.7	4.55	4.35	4.4	**Treat**	**0.0554**
mmol/l		**Min - Max**	3.6 - 5.3	3.4 - 5.2	3.9 - 5.6	3.7 - 5.2	4.0 - 6.2	4.0 - 6.5	3.9 - 5.2	3.9 - 5.0	3.5 -5.3	**Sample**	**0.1021**
		N	27	28	28	28	28	28	28	28	27	**Treat * Sample**	**0.0001**
		Compare to Sample 1		**P = 0.5000**			**P = 0.0726**			**P = 0.8131**			
	Transport (T)	**Median**	4.5	4.9	4.4	4.5	4.8	4.25	4.6	4.6	5.1		
		Min - Max	3.4 - 9.6	3.7 - 10.5	3.6 - 5.7	4.0 - 6.0	4.0 - 6.7	3.5 - 5.9	3.8 - 5.7	3.6 - 5.9	4.2 - 6.9		
		N	52	51	51	51	52	52	52	52	52		
		Compare to Sample 1		**0.0021**			**0.8000**			**0.0820**			
		Sig. (C v's T)	**P = 0.2923**	**P = 0.0001**	**P = 0.052**	**P = 0.1893**	**P = 0.0057**	**P = 0.0014**	**P = 0.2692**	**P = 0.042**	**P = 0.0001**		
Normal range	2.50 – 4.16 mmol/l	(3.19 ± 0.38)	Kaneko, 1989										

* after 12 hours; ** after 24 hours

Table 17: Plasma haptoglobin concentrations in control and transported animals (samples 1 to 9). Pre-transport measurements were taken at –24 hours (day 0). ***Values are expressed as Median with minimum and Maximum values*** **with P values.**

			Pre-transport	Arrival in French lairage	Lairage *	Lairage ** Depart for Spain	Spanish Feedlot	Feedlot	Feedlot	Feedlot	Feedlot		
		Day	0	2	3	3	5	7	9	11	38		
Variable	**Treatment**	**Statistic**	**Sample 1**	**Sample 2**	**Sample 3**	**Sample 4**	**Sample 5**	**Sample 6**	**Sample 7**	**Sample 8**	**Sample 9**	**Repeated**	**Sig. P =**
HAPTOGLOBIN	**Control (C)**	**Median**	0.23	0.24	0.19	0.23	0.205	0.22	0.21	0.2	0.18	**Treat**	**0.0001**
g Hb-binding		**Min - Max**	0.14 - 1.67	0.19 - 2.74	0.04 - 2.35	0.18 - 1.89	0.14 - 0.97	0.16 - 0.80	0.13 - 1.27	0.14 - 0.87	0.15 - 0.31	**Sample**	**0.4664**
capacity/l		**N**	28	28	28	28	28	28	28	28	27	**Treat * Sample**	**0.0001**
		Compare to Sample 1		**P = 0.1457**			**P = 0.6094**			**P = 0.3019**			
	Transport (T)	**Median**	0.22	0.31	0.38	0.255	0.3	0.28	0.42	0.22	0.205		
		Min - Max	0.12 - 1.09	0.19 - 3.37	0.20 - 3.44	0.14 - 2.71	0.19 - 4.74	0.15 - 4.89	0.16 - 3.86	0.12 - 1.78	0.15 - 1.02		
		N	52	52	52	52	52	51	51	52	52		
		Compare to Sample 1		**P = 0.0002**			**P = 0.0031**			**P = 0.0027**			
		Sig. (C v's T)	**P = 0.1507**	**P = 0.0266**	**P = 0.0001**	**P = 0.7655**	**P = 0.0001**	**P = 0.0041**	**P = 0.0001**	**P = 0.5137**	**P = 0.0047**		
Normal range													

Table 18: Plasma fibrinogen concentrations in control and transported animals (samples 1 to 9). Pre-transport measurements were taken at –24 hours (day 0). ***Values are expressed as Median with minimum and Maximum values*** **with P values.**

Variable	**Treatment**	**Statistic**	**Sample 1**	**Sample 2**	**Sample 3**	**Sample 4**	**Sample 5**	**Sample 6**	**Sample 7**	**Sample 8**	**Sample 9**	**Repeated**	**Sig. P =**
FIBRINOGEN	**Control (C)**	**Mean**	558.4	514.1	560.7	582.6	560.6	687.2	654.3	692.4	446.8	**Treat**	**0.6260**
mg/dl		**SD**	185.27	125.64	147.77	155.55	118.32	138.99	147.96	114.12	83.71	**Sample**	**0.0001**
		N	27	28	28	28	28	28	28	28	27	**Treat * Sample**	**0.0035**
		Compare to Sample 1		**P = 0.0787**			**P = 0.9059**			**P = 0.0016**			
	Transport (T)	**Mean**	486.2	629.6	565	555.6	614.7	731.9	648.9	638.8	488.4		
		SD	181.29	199.48	172.71	194.95	225.46	310.82	265.51	186.9	112.14		
		N	52	52	51	51	52	51	50	52	52		
		Compare to Sample 1		**P = 0.0001**			**P = 0.0001**			**P = 0.0001**			
		Sig. (C v's T)	**P = 0.1147**	**P = 0.0057**	**P = 0.9346**	**P = 0.4808**	**P = 0.2271**	**P = 0.3597**	**P = 0.8433**	**P = 0.2729**	**P = 0.0728**		
Normal range	300-700 mg/dl	Kaneko, 1989											

*** after 12 hours; ** after 24 hours**

Table 19: Plasma lactate concentrations in control and transported animals (samples 1 to 9). Pre-transport measurements were taken at –24 hours (day 0). *Values are expressed as Median with minimum and Maximum values* with P values.

			Pre-transport	Arrival in French lairage	Lairage *	Lairage ** Depart for Spain	Spanish Feedlot	Feedlot	Feedlot	Feedlot	Feedlot		
		Day	0	2	3	3	5	7	9	11	38		
Variable	**Treatment**	**Statistic**	**Sample 1**	**Sample 2**	**Sample 3**	**Sample 4**	**Sample 5**	**Sample 6**	**Sample 7**	**Sample 8**	**Sample 9**	**Repeated**	**Sig. P =**
LACTATE	**Control (C)**	**Median**	3.56	2.365	2.265	1.715	1.47	1.69	1.58	1.585	2.49	**Treat**	**0.0805**
mmol/l		**Min - Max**	0.78 - 8.31	0.79 - 6.9	0.69 - 5.7	0.81 - 5.35	0.67 - 3.5	0.71 - 3.14	0.66 - 4.92	0.64 - 4.83	1.1 - 5.86	**Sample**	**0.9011**
		N	27	28	28	28	28	28	28	28	27	**Treat * Sample**	**0.0001**
		Compare to Sample 1		**P = 0.0102**			**P = 0.0001**			**P = 0.0001**			
	Transport (T)	**Median**	3.725	4.045	1.99	1.7	2.705	2.145	2.095	1.945	1.96		
		Min - Max	1.27 - 23.52	1.11 - 18.82	0.7 - 8.44	0.59 - 6.36	0.88 - 8.33	0.65 - 8.3	0.83 - 8.34	0.79 - 6.95	0.66 - 9.16		
		N	52	52	51	51	52	52	52	52	52		
		Compare to Sample 1		**P = 0.3443**			**P = 0.0005**			**P = 0.0001**			
		Sig. (C v's T)	**P = 0.7668**	**P = 0.0428**	**P = 0.6432**	**P = 0.9334**	**P = 0.0001**	**P = 0.0362**	**P = 0.0194**	**P = 0.0588**	**P = 0.0643**		
Normal range	0.56 – 2.22	Kaneko, 1989											

Table 20: Plasma non-esterified fatty acid (NEFA) concentrations in control and transported animals (samples 1 to 9). Pre-transport measurements were taken at –24 hours (day 0). *Values are expressed as Median with minimum and Maximum values* with P values.

Variable	**Treatment**	**Statistic**	**Sample 1**	**Sample 2**	**Sample 3**	**Sample 4**	**Sample 5**	**Sample 6**	**Sample 7**	**Sample 8**	**Sample 9**	**Repeated**	**Sig. P =**
NEFA	**Control (C)**	**Median**	0.23	0.21	0.225	0.2	0.305	0.32	0.255	0.24	0.31	**Treat**	**0.0001**
µmol/l		**Min - Max**	0.01 - 0.64	0.01 - 0.50	0.14 - 0.83	0.13 - 0.66	0.01 - 1.19	0.15 - 0.81	0.11 - 1.29	0.01 - 1.28	0.12 - 0.78	**Sample**	**0.0835**
		N	27	28	28	28	28	28	28	28	27	**Treat * Sample**	**0.0001**
		Compare to Sample 1		**P = 0.1808**			**P = 0.0336**			**P = 0.2302**			
	Transport (T)	**Median**	0.22	0.68	0.67	0.585	1.135	0.65	0.6	0.495	0.225		
		Min - Max	0.01 - 0.88	0.01 - 1.87	0.01 - 1.30	0.16 - 1.02	0.01 - 2.11	0.01 - 1.64	0.19 - 1.61	0.01 - 1.56	0.12 - 0.40		
		N	52	51	51	50	52	52	52	52	52		
		Compare to Sample 1		**P = 0.0001**			**P = 0.0001**			**P = 0.0001**			
		Sig. (C v's T)	**P = 0.9859**	**P = 0.0001**	**P = 0.0001**	**P = 0.0001**	**P = 0.0001**	**P = 0.0001**	**P = 0.0001**	**P = 0.0001**	**P = 0.0001**		
Normal range	0-600	(Knowles et al., 2000)											

*** after 12 hours; ** after 24 hours**

Table 21: Plasma urea concentrations in control and transported animals (samples 1 to 9). Pre-transport measurements were taken at –24 hours (day 0). *Values are expressed as Median with minimum and Maximum values* with P values.

			Pre-transport	Arrival in French lairage	Lairage *	Lairage ** Depart for Spain	Spanish Feedlot	Feedlot	Feedlot	Feedlot	Feedlot		
		Day	0	2	3	3	5	7	9	11	38		
Variable	**Treatment**	**Statistic**	**Sample 1**	**Sample 2**	**Sample 3**	**Sample 4**	**Sample 5**	**Sample 6**	**Sample 7**	**Sample 8**	**Sample 9**	**Repeated**	**Sig. P =**
UREA	**Control (C)**	**Median**	3.85	3.6	3.6	3.1	3.9	3	3.25	3.15	4.5	**Treat**	**0.0001**
mmol/l		**Min - Max**	2.5 - 5.6	2.0 - 5.3	2.1 - 4.8	1.5 - 4.6	1.9 - 5.6	1.5 - 4.7	1.8 - 5.3	2.1 -3.9	2.7 - 7.8	**Sample**	**0.589**
		N	28	28	27	28	28	28	28	28	27	**Treat * Sample**	**0.0001**
		Compare to Sample 1		**P = 0.2832**			**P = 0.5714**			**P = 0.0001**			
	Transport (T)	**Median**	4.4	6.2	5.2	3.65	3.8	3.2	3.25	3.55	5.5		
		Min - Max	2.1 - 7.1	3.5 - 9.5	2.8 - 8.9	1.7 - 5.3	2.1 - 5.9	1.6 - 6.1	2.3 - 5.8	1.8 - 6.2	2.8 - 7.6		
		N	52	52	52	52	52	51	48	52	52		
		Compare to Sample 1		**P = 0.0001**			**P = 0.0003**			**P = 0.0014**			
		Sig. (C v's T)	**P = 0.101**	**P = 0.0001**	**P = 0.0001**	**P = 0.0537**	**P = 0.8151**	**P = 0.7437**	**P = 0.7527**	**P = 0.0939**	**P = 0.0008**		
Normal range	7.14 – 10.7 mmol/l	Kaneko, 1989											

Table 22: Plasma beta-hydroxy butyrate (BHB) concentrations in control and transported animals (samples 1 to 9). Pre-transport measurements were taken at –24 hours (day 0). *Values are expressed as Mean ± SD* with P values.

Normal Data - Repeated Measures Analysis of Variance													
Variable	**Treatment**	**Statistic**	**Sample 1**	**Sample 2**	**Sample 3**	**Sample 4**	**Sample 5**	**Sample 6**	**Sample 7**	**Sample 8**	**Sample 9**	**Repeated**	**Sig. P =**
BHB	**Control (C)**	**Mean**	0.31	0.41	0.29	0.39	0.24	0.22	0.24	0.29	0.24	**Treat**	**0.0795**
g/l		**SD**	0.095	0.161	0.122	0.117	0.093	0.071	0.074	0.075	0.071	**Sample**	**0.0001**
		N	28	28	27	28	28	28	28	28	27	**Treat * Sample**	**0.0001**
		Compare to Sample 1		**P = 0.0003**			**P = 0.0060**			**P = 0.5179**			
	Transport (T)	**Mean**	0.23	0.26	0.31	0.26	0.33	0.31	0.22	0.21	0.19		
		SD	0.1	0.197	0.087	0.085	0.097	0.176	0.1	0.086	0.06		
		N	52	52	52	52	52	51	48	52	52		
		Compare to Sample 1		**P = 0.3166**			**P = 0.0001**			**P = 0.1278**			
		Sig. (C v's T)	**P = 0.0214**	**P = 0.0055**	**P = 0.2571**	**P = 0.0001**	**P = 0.0002**	**P = 0.0094**	**P = 0.3374**	**P = 0.0001**	**P = 0.0023**		
Normal range	0.00 – 1.2	(Knowles et al., 2000)											

*** after 12 hours; ** after 24 hours**

Table 23: Plasma protein concentrations in control and transported animals (samples 1 to 9). Pre-transport measurements were taken at –24 hours (day 0). *Values are expressed as Mean ± SD* with P values.

			Pre-transport	Arrival in French lairage	Lairage *	Lairage ** Depart for Spain	Spanish Feedlot	Feedlot	Feedlot	Feedlot	Feedlot		
		Day	0	2	3	3	5	7	9	11	38		
Variable	**Treatment**	**Statistic**	**Sample 1**	**Sample 2**	**Sample 3**	**Sample 4**	**Sample 5**	**Sample 6**	**Sample 7**	**Sample 8**	**Sample 9**	**Repeated**	**Sig. P =**
PROTEIN	**Control (C)**	**Mean**	75.87	73.4	74.3	73.23	73.16	73.73	72.38	71.35	70.24	**Treat**	**0.0001**
g/l		**SD**	4.768	4.09	4.191	4.303	4.172	4.327	3.279	4.141	3.887	**Sample**	**0.0001**
		N	28	28	27	28	28	28	28	28	27	**Treat * Sample**	**0.0001**
		Compare to Sample 1		**P = 0.0001**			**P = 0.0001**			**P = 0.0001**			
	Transport (T)	**Mean**	75.89	80.88	76.66	78.3	77.67	77.72	76.24	76.26	75.34		
		SD	5.969	4.792	4.859	5.189	5.219	8.163	5.958	5.29	4.626		
		N	52	52	52	52	52	51	48	52	52		
		Compare to Sample 1		**P = 0.0001**			**P = 0.0015**			**P = 0.5408**			
		Sig. (C v's T)	**P = 0.7176**	**P = 0.0001**	**P = 0.0159**	**P = 0.0001**	**P = 0.0001**	**P = 0.0091**	**P = 0.002**	**P = 0.0001**	**P = 0.0001**		
Normal range	67.4 – 74.6 g/l	71.0 ± 1.8	Kaneko, 1989										

Table 24: Plasma LDH concentrations in control and transported animals (samples 1 to 9). Pre-transport measurements were taken at –24 hours (day 0). *Values are expressed as Mean ± SD* with P values.

Variable	**Treatment**	**Statistic**	**Sample 1**	**Sample 2**	**Sample 3**	**Sample 4**	**Sample 5**	**Sample 6**	**Sample 7**	**Sample 8**	**Sample 9**	**Repeated**	**Sig. P =**
LDH	**Control (C)**	**Mean**	2625.7	2478.7	2651.1	2410.3	2283	1946.5	2042	2024	2566.5	**Treat**	**0.0073**
U/L		**SD**	396.69	378.51	413.45	374.14	351.6	399.67	367.03	254.57	392.8	**Sample**	**0.0001**
		N	28	28	27	28	28	28	28	28	27	**Treat * Sample**	**0.0001**
		Compare to Sample 1		**P = 0.0008**			**P = 0.0001**			**P = 0.0001**			
	Transport (T)	**Mean**	2520.2	2758.5	2740.8	2580.8	2425.6	2873.6	2303.2	2319.3	2502.5		
		SD	360.24	441.31	546	441.99	410.22	831.13	424.85	402.21	352.65		
		N	52	52	52	52	52	51	48	52	52		
		Compare to Sample 1		**P = 0.0001**			**P = 0.0823**			**P = 0.0011**			
		Sig. (C v's T)	**P = 0.2161**	**P = 0.0095**	**P = 0.5206**	**P = 0.1505**	**P = 0.1654**	**P = 0.0001**	**P = 0.0091**	**P = 0.0017**	**P = 0.476**		
Normal range	692-1445	(1061 ± 222)	Kaneko, 1989										

- **after 12 hours; ** after 24 hours**

Table 25: Plasma cortisol concentrations in control and transported animals (samples 1 to 9). Pre-transport measurements were taken at –24 hours (day 0). *Values are expressed as Mean ± SD* with P values.

			Pre-transport	Arrival in French lairage	Lairage *	Lairage ** Depart for Spain	Spanish Feedlot	Feedlot	Feedlot	Feedlot	Feedlot		
		Day	0	2	3	3	5	7	9	11	38		
Variable	**Treatment**	**Statistic**	**Sample 1**	**Sample 2**	**Sample 3**	**Sample 4**	**Sample 5**	**Sample 6**	**Sample 7**	**Sample 8**	**Sample 9**	**Repeated**	**Sig. P =**
Cortisol	**Control (C)**	**Mean**	17.812	25.831	18.786	19.463	17.773	20.979	14.213	13.485	33.382	**Treat**	**0.9444**
ng/ml		**SD**	9.1002	12.8821	10.7499	9.8327	9.0144	10.2283	9.869	8.1655	17.1343	**Sample**	**0.0001**
		N	28	28	28	28	28	28	28	28	28	**Treat * Sample**	**0.0004**
		Compare to sample 1		**P = 0.0037**			**P = 0.9878**			**P = 0.0879**			
	Transport (T)	**Mean**	21.692	26.202	14.322	12.194	22.052	18.769	18.111	18.064	30.231		
		SD	13.8477	15.7634	7.7869	8.2047	9.2753	8.6919	9.679	9.2496	14.9643		
		N	52	52	52	52	52	52	52	52	52		
		Compare to sample 1		**P = 0.0441**			**P = 0.8616**			**P = 0.0611**			
		Sig. (C v's T)	**P = 0.1851**	**P = 0.9332**	**P = 0.0377**	**P = 0.0014**	**P = 0.0628**	**P = 0.3712**	**P = 0.1325**	**P = 0.0455**	**P = 0.2941**		

*** after 12 hours; ** after 24 hours**

8. Conclusion

There was little evidence that transport affected physiological, haematological and immunological variables in the present study, and there was no evidence to suggest that it adversely affected the health or the performance of the animals post transport.

Animals transported to France lost 7.6 % of their bodyweight, and gained 3.3 % of their bodyweight by time of arrival in Spain and recovered to pre-transport liveweight values by day 6. There was some evidence that transport affected physiological and immunological variables, there was no evidence to suggest that it adversely affected the health or the performance of the animals post transport.

Creatine kinase activities were increased but values were still within normal acceptable ranges. Increases in non-esterified fatty acids, βeta-hydroxybutyrate and urea concentrations suggested that the animals' normal pattern of feeding was disrupted during transport. Increases in albumin, total plasma protein and osmolality would indicate slight dehydration during transit. However, albumin concentrations returned to control levels by day 38 of the study. While haematocrit values were decreased, they are within the range of normal referenced data (24 - 48%). Similarly, changes in the red blood cell numbers and haemoglobin were within the normal blood referenced ranges. The aspartate transaminase concentrations for the transported animals at arrival in France and Spain were not significantly different from their pre-transport concentrations but were increased at day 11 when compared with baseline levels.

Concanavalin-A induced interferon-γ levels were lower on arrival in the Spanish feedlot and on Day 11 of the study, when compared with pre-transport baseline levels. Compared with pre-transport levels, keyhole limpet haemocyanin-induced interferon-γ levels for the transported animals were significantly decreased on the day of arrival in France, with no significant difference on the day of arrival in Spain or on day 11 of the study. Interferon-γ is produced by activated T lymphocytes and natural killer cells in response to antigen. The percentage (%) of lymphocytes decreased and the % neutrophils increased post-transport indicating a shift in the population of these blood cells relative to pre-transport baseline values. There was no significant change in plasma cortisol concentrations in transported animals at arrival in France and in Spain. On Day 11, the plasma cortisol concentrations of transported animals were significantly higher than control animals.

There were significantly higher glucose concentrations on arrival in France, and in samples taken at 12 and 24 hours post-arrival in France, on arrival in Spain, and on days 7 and 11 compared with control levels. Transported animals had significantly higher glucose levels at sample 2 on the day of arrival in France compared with their pre-transport values. Transported animals had significantly higher fibrinogen levels at arrival in France compared with their pre-transport baseline concentrations. Transported animals had significantly higher non-esterified fatty acid (NEFA) levels on arrival in France and Spain and on day 11 compared with their pre-transport baseline concentrations. Control animals had significantly higher levels on day 5 compared with their pre-transport baseline NEFA concentrations.

Physiological, haematological and immunological variables are used to determine the welfare status of animals. Several studies have been conducted to determine the short-term effects of transport and associated factors (e.g. loading, journey duration) on calf welfare (Knowles *et al.*, 1997; Murata *et al.*, 1985 Todd *et al.*, 2000; Blecha *et al.*, 1984; Mormede *et al.*, 1982, Staples and Hague, 1974). Most of these studies have shown a transient acute physiological response to transport and handling (characterised by increased cortisol concentrations) along with other biological responses which are related mainly to the duration of food and water deprivation. Warriss et al. (1995) transported steers by road that were 12- to 18-mo-old, for either 5, 10 or 15 h. There were no differences in environmental temperatures experienced by cattle on the three treatments.

Warriss et al. (1995) reported that animals that were transported for 5, 10 and 15 h lost 4.6, 6.5 and 7.0% of their bodyweight, respectively; and recovery to pre-transport BW generally took 5 days. Only plasma creatine phosphokinase concentrations increased with journey length, although plasma creatine phosphokinase, urea and albumin concentrations and plasma osmolality took longer to recover after longer journeys. Warriss et al. (1995) concluded that under the conditions of their study, a 15-h journey by road for 12- to 18-mo-old cattle did not impact unacceptably on the welfare of the animals. One of the concerns regarding transportation of cattle is that any resultant stress may be immunosuppressive and render the animals more susceptible to disease.
The study concluded that transport had no adverse effect on animal welfare based on the physiological, immunological and haematological measurements made.

9. Acknowledgments

The study was undertaken in collaboration with Dr. Malcolm Mitchell (Roslin, UK), Mr. Peter Kettlewell (Silsoe, UK), Dr. Valerie Fessard, Agence Française de Sécurité Sanitaire des Aliments ((AFSSA, Fougeres, France)), Dr. Pascal Sanders ((AFSSA, Fougeres, France)), Dr. Jean-Michel Poul ((AFSSA, Fougeres, France)), Dr. Julio de la Fuente Martinex, Instituto Nacional de Investigacion Y Tecnologia Agraria Y Alimentaria (INIA Madrid), Dr. Belen Pintado (INIA Madrid), Dr. Concha Borque (INIA Madrid).

The authors gratefully acknowledge the technical assistance of: Francis Collier, Padraig Gormley, Joe Larkin, Ann Marley, Mary Munnelly, Joe Munroe, Liam Moore, Michael Nolan, Julianne Price, Simon Perry, Dan Prendiville and Jim Robinson. Many thanks are due to: the farm foreman, Gerry Santry, farm staff – Joe Gill, John Horan, Eddie Mulligan, Hugh Mulligan, Paschal Reilly, for their assistance throughout the experiment; to Ann Gilsenan, for typesetting and graphics.

10. References

Andersson, B.E. and Jonasson, H., Temperature Regulation and Environmental Physiology, in *Duke's Physiology of Domestic Animals*, 11th ed., Swenson M.J. and Reece W.O., Eds., 1993, used by permission of the publisher, Cornell University Press.

Blecha, F., S. L. Boyles, and J. G. Riley. (1984). Shipping suppresses lymphocyte blastogenic responses in Angus and Brahman, Angus feeder calves. J. Anim. Sci. 59:576-583.

Earley, B. and M.A.. Crowe. (2002). Effects of ketoprofen alone or in combination with local anesthesia during castration of bull calves on plasma haptoglobin, *in-vitro* interferon-γ production, white blood cell numbers and animal performance. J. Anim. Sci. 80:1044-1052.

Jain, N.C. (1986) Schalm's Veterinary haematology, 4th Edition, Philadelphia, Lea &Fibiger.

Kaneko, J.J. (1989) Clinical Biochemistry of Domestic animals. 4th Edition, Academic Press Inc., San Diego, pp886-891.

Kenny, F. J. and P. V. Tarrant. (1987a). The behaviour of young Friesian bulls during social re-grouping at an abbatoir. Influence of an overhead electrified wire grid. Appl. Anim. Behav. Sci. 18: 233-246.

Kenny, F. J. and P. V. Tarrant. (1987b).The physiological and behavioural responses of crossbred fresian steers to short-haul transport by road. Livest. Prod. Sci. 17: 63-75.

Knowles, T.G., Edwards, J.E., Bazeley, K.J., Brown, S.N., Butterworth, A. and P.D. Wariss (2000). Changes in the blood biochemical and haematological profile of neonatal calves with age.

Knowles, T.G., Warriss, P.D., Warriss, S.N., Brown, J.E., Edwards, P.E. and A.J. Phillips (1997). Effects on calves less than one month old of feeding or not feeding them during road transport of up to 24 hours. Veterinary Record, 140, 116-124.

Mormede, P., Soissons, J., Bluthe, R.M., Raqult, J., Legarff, G., Levieux, D. and Dantzer, R. (1982). Effect of transportation on blood serum composition, disease incidence, and production traits in young calves influence of the journey duration. Ann. Rech. Vet., 13 (4) 369-384.

Murata, H., H. Takahashi and H. Matsumoto. (1985). Influence of truck transportation of calves in their cellular immune function. Jpn. J. Vet. Sci. 47: 823-827.

Radostits, O.M., Blood, D.C., and C.C. Gay. (1994) Veterinary Medicine: a textbook of the diseases of cattle, sheep, Pigs, Goats and horses. London, Balliere Tindall.

SAS/STATISTIC® software Version 6.1 of the SAS System for Windows. Copyright© 1989-1996 SAS Institute Inc. SAS and all other SAS Institute Inc. product or service names are registered trademarks or trademarks of SAS Institute Inc. Cary, NC, USA.

Schalm, O.W. (1961) Balliere Tindall and Cox, Covent Garden, London, UK. pp130-131.

Schalm, O.W. (1984) Manual of bovine haematology: Anemias/leukocytes/testing. Santa Barbara, Veterinary Practice Publishing.

Staples, G.E. and C.N. Hauge (1974). Losses in young calves after transportation. British Veterinary Journal, 130, 374.

Todd ,S.E, Mellor, D.J., Stafford, K.J., Gregory, N.G., Bruce, R.A., and R.N. Ward, (2000). Effects of food withdrawal and transport on 5-to 10-day-old calves, Research in Veterinary Science. 68: (2) 125-134.

Warriss, P. D., S. N. Brown, T. G. Knowles, S. C. Kestin, J. E. Edwards, S. K. Dolan, and A. J. Phillips. (1995). Effects on cattle of transport by road for up to 15 hours. Vet. Rec. 136:319-323.

Experiment 2:

The physiological, haematological and immunological responses of 9-month old bulls (250kg) to transport at two stocking densities (0.85m^2 and 1.27m^2 /250kg animal) on a 12-hour journey by road.

Authors

Bernadette Earley, Joseph A. Farrell, Margaret Murray,
Dan Prendiville, Edward G. O'Riordan

Teagasc, Grange Research Centre, Dunsany, Co. Meath, Ireland

2003
Teagasc
Grange Research Centre
Dunsany
Co. Meath
Ireland

Contents **Page No**

1. Summary

This report describes the result of a study designed to investigate the physiological, haematological and immunological responses of 9-month old bulls (250kg) to transport at two stocking densities (0.85m^2 and 1.27m^2 /250kg animal) on a 12-hour journey by road. The results indicate that within the conditions of the study, that there was no welfare advantage in transporting bulls at 1.27m^2 versus the standard stocking density of 0.85 m^2 on a 12 hour road journey.

Protein, globulin, urea and lactate concentrations, and white blood cell numbers were not significantly changed at any time during the experiment. The activities of the enzymes creatine kinase, aspartate aminotransferase and lactate dehydrogenase were not altered by transportation at either the 0.85 or 1.27 m^2 stocking densities. Following transportation all transported groups had significantly higher albumin levels than the control animals.

There was no significant difference between treatments in beta-hydroxybutyrate concentrations (BHB) prior to transport. BHB concentrations were significantly decreased in all animals following transport. Pre-transport non-esterified fatty acid (NEFA) concentrations were not significantly different. Following transport, animals transported at a stocking density of 1.27m^2 had significantly higher NEFA concentrations compared with control values. There were no significant differences in glucose concentrations between treatments prior to transport. Post-transport, blood glucose concentrations were significantly higher in all transported animals compared with control values.

The % lymphocytes were reduced in the transported animals post-transport and there was no significant change in lymphocyte numbers. The % of neutrophils and the number of neutrophils were significantly increased in all transported animals. There were no significant change in monocyte numbers, % monocytes and platelet numbers following transportation. The haematocrit values and red blood cell (RBC) numbers were significantly higher in the transported bulls. However, haematocrit % for the animals at 1.27m^2 were significantly higher than the control animals prior to transport. RBC numbers were similar prior to transport. RBC numbers were higher in the animals transported at a stocking density of 1.27m^2 compared with control. Haemoglobin levels for the animals at 1.27m^2 were significantly higher than the control animals prior to transport and after transport.

There were no significant differences in the stimulated production of interferon-γ in response to concanavalin-A (Con-A) and keyhole limpet haemocyanin (KLH), and cortisol between treatments prior to or after transport. Plasma haptoglobin concentrations were unchanged following transportation while plasma fibrinogen levels were significantly reduced in the two transported groups. There was no significant difference in rectal body temperature, pre and post transport. There were no significant differences in liveweight between the control and transported groups before the journey or on Day 8 after the journey. There was no significant difference in the rate of gain for either control or transported animals at either the 0.85m^2 or the 1.27m^2 stocking densities.

2. Introduction

The Scientific Committee on Animal Health and Welfare (SCHAW), advising the European Commission recently, (March 2002) adopted a report on the welfare of animals during transport. The scientists concluded that both welfare and health of animals can be substantially affected in the course of and as a result of transport. The Committee advised on maximum travel and resting times, watering and feeding intervals, stocking densities and loading methods. It also advised that the transport of very young animals should be prohibited. The Committee stressed the importance of proper training for the personnel responsible for animals during transport. The scientific opinion is now being examined by the Commission.

The recent SCAHAW report showed that the scientific basis for several of the EU regulations (e.g. EC 91/628; EC 98/411) is weak and where there are data, there are different opinions regarding conclusions to be drawn. A clear disadvantage is that the recommendations are 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 will not include the extremes of climate possible within Europe. Furthermore, there is no scientific data on which to base guidelines for stocking density, as most are based on the animals' size and on practical experience. There are, for example, referenced investigations on the effects of stocking density during road transport; Eldridge *et al.* (1988) transported heifers (350 kg BW) at 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 allowances had lower heart rates and movement scores, and Eldridge *et al.* (1988) speculated 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 results and conclusions of Tarrant *et al.* (1988) and Tarrant *et al.* (1992). Tarrant *et al*. (1988) transported steers (603 kg BW) at space allowances of 1.02, 1.93 and 3.0 m^2/animal. Plasma cortisol, creatine kinase, muscle bruising observed at slaughter, and the incidence of animals falling during transport and being unable to rise, all increased with decreasing space allowance. Cattle preferred to orient themselves parallel to the direction of travel at greater space allowances. Tarrant *et al.* (1992) used space allowances of 1.03 to 1.08, 1.19 to 1.24 and 1.33 to 1.41 m^2/animal to transport steers of 600 kg BW, with a series of three journeys. Steers transported at the lowest space allowance had the highest incidence of falls and struggles to maintain balance, whereas at the medium and high space allowances, animals were more often observed to be able to shift position to maintain balance. Losses of balance were also more common among animals situated to the rear of the truck. However, this was most likely to happen with old transporters and may have no relevance to the modern air spring designs. Plasma cortisol and creatine kinase and carcass bruising were all increased by reduced space allowance. Tarrant *et al.* (1992) concluded that space allowances similar to the lowest used in the study were detrimental to the welfare of transported cattle. Penning conditions and stocking density within transport vehicles have been shown to affects the responses of cattle to transport.

Lambooy and Hulsegge (1988) transported heifers (476 to 533 kg BW) by truck in loose pens or penned in pairs, at similar stocking densities (1.4 to 1.7 m^2/animal). A series of 5 journeys were made, each of 25 h duration, incorporating two 1-h rest stops and a 3-h stop when the heifers were watered and fed on board the vehicle. Penning conditions had no effect on biochemical variables, including packed cell volume, ketone and glucose concentrations. While loose-penned heifers lost more bodyweight during transport, 10 out of 40 heifers that were transported and penned in pairs suffered skin lesions and injury.

The Farm Animal Welfare Council (FAWC)(UK) produced the formula $\mathbf{A = 0.021\ W^{0.67}}$ for calculating the minimal spatial area, in m^2, for each animal based on liveweight:, where A = the area

in square metres and W = liveweight (Kg). Using published guidelines for stocking density from other sources, Randall (1993) derived the equation where A= 0.01 $W^{0.78}$; however, that author recommended the use of this equation given by FAWC because it was more generous in its space allowance for larger animals. The recent SCHAW report (2002) (page 99) recommends 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".

The overall objective of the present study was to investigate the physiological, haematological and immunological responses of 9-month old bulls (250kg) to transport at the standard stocking rate of 0.85m^2 (old) and the revised stocking rate 1.27m^2 (new) on a 12-hour journey by road.

3. Objectives

3. To quantify the effects of transport at two different stocking densities on the degree of stress imposed and the ability of the bulls to cope with that stress of transport.
4. To make appropriate physiological and environmental measurements on the bulls prior to and after transport.

4. Materials and methods

The 12-hour transport was carried out in July 2002. Twenty-nine bulls were transported by road on a 12 hour journey while 16 control bulls were housed on slats (2m^2/animal) and fed *ad lib* silage and 2 kg of concentrates at Grange Research Centre, Co. Meath. The bulls were transported on the bottom of an articulated transporter (total area = 30.96m^2) which was divided into 4 pens with the following dimensions:

Articulated transporter			
Pen	Length	Width	Area m^2
1	**3.9**	**2.4**	**9.36**
2	**3**	**2.4**	**7.2**
3	**3**	**2.4**	**7.2**
4	**3**	**2.4**	**7.2**
Total area			**30.96**

On the evening of the journey (July 8th, 2002), all animals were blood sampled (day 0; Sample 1) by jugular venepuncture to provide baseline physiological values. The bulls were weighed and randomly allocated, at 18:00h, to 4 fan ventilated pens on an animal transporter at a stocking density of either 1.27 m^2 (N = 13) and 0.85 m^2 (N = 16) per animal and transported on a 12-hour journey by road. The individual pens on the transporter were bedded with sawdust and water was available through nipple drinkers. The 16 bulls remaining at Grange Research Centre were housed in a slatted shed at a standard stocking density of 2m^2 per animal served as control animals. The control bulls were blood sampled and weighed at times corresponding to the transported animals.

The 12 hour journey from Grange Research Centre to Co. Cork and return (608km), involved a combination of road surfaces ranging from motorways, secondary roads to small country lanes. On completion of the 12-hour journey, blood samples were collected by jugular venepuncture (Sample2) for physiological and haematological measurements.

5. Body temperature (Rectal, deep-body, and surface)

Rectal temperatures were recorded using a digital thermometer (Jorgen Kruuse A/S; Model VT-801BWC Lot No 0701) prior to transportation on day 0 and days 2, 3, 5, 7, 9, 11 and 38 of the study.

Rumen boluses (Cow Temp^{TM})(Innotek, Indiana, US) were inserted into the rumen of 12 animals (8 transport and 4 control) 4 hours prior to transport and were used to monitor deep body temperature before and during the transport journey.

The surface body temperatures (^{0}C) (shoulder, rump, belly) of all animals was recorded using a hand help laser device (Raytek MX series 16 point laser, Radionics, Dublin, Ireland) on completion of the 12-hour journey.

6. Physiological, haematological and immunological variables.

Blood samples collected by jugular venepuncture and placed into heparinised tubes were centrifuged and the plasma separated for subsequent analysis of: cortisol, glucose, lactate, free fatty acids, βeta-hydroxy butyrate (βHB), urea, total protein, albumin, creatine phosphokinase (CPK), lactate dehydrogenase (LDH), and the acute phase proteins (fibrinogen and haptoglobin. Blood samples for interferon-γ determination were collected by jugular venepuncture into aseptic vacutainer tubes containing lithium heparin and the stimulated lymphocyte production of interferon-γ was determined following whole blood culture of heparinised samples using an 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 (TWC) count, % granulocytes, % monocytes, platelet number, percentage lymphocytes) were determined for unclotted (K_3-EDTA) whole blood samples using an electronic particle hematology analyser (Celltac MEK-610K, Nihon Kohden, Japan and a blood haematology analyser). Plasma cortisol concentrations were determined using a commercially available RIA kit. Plasma haptoglobin concentrations were measured by determining the haemoglobin-binding capacity using a biochemical autoanalyser. Fibrinogen concentrations are measured using a commercial biochemical assay kit (Boehringer Mannheim, Germany). All other physiological measurements were made using Randox assay procedures.

Electricity was supplied to operate the computerised equipment for monitoring environmental conditions during transport on the transporter using a 10Kw generator (Lister Petter LPW3ssd-A10). H_2S and NH_3 were measured by Q Rae (ShawcityLtd UK) plus confined space detector kit temp. Relative humidity, CO_2 and wind velocity measured by Testo 445 portable multifunction probes (Testo UK Ltd).

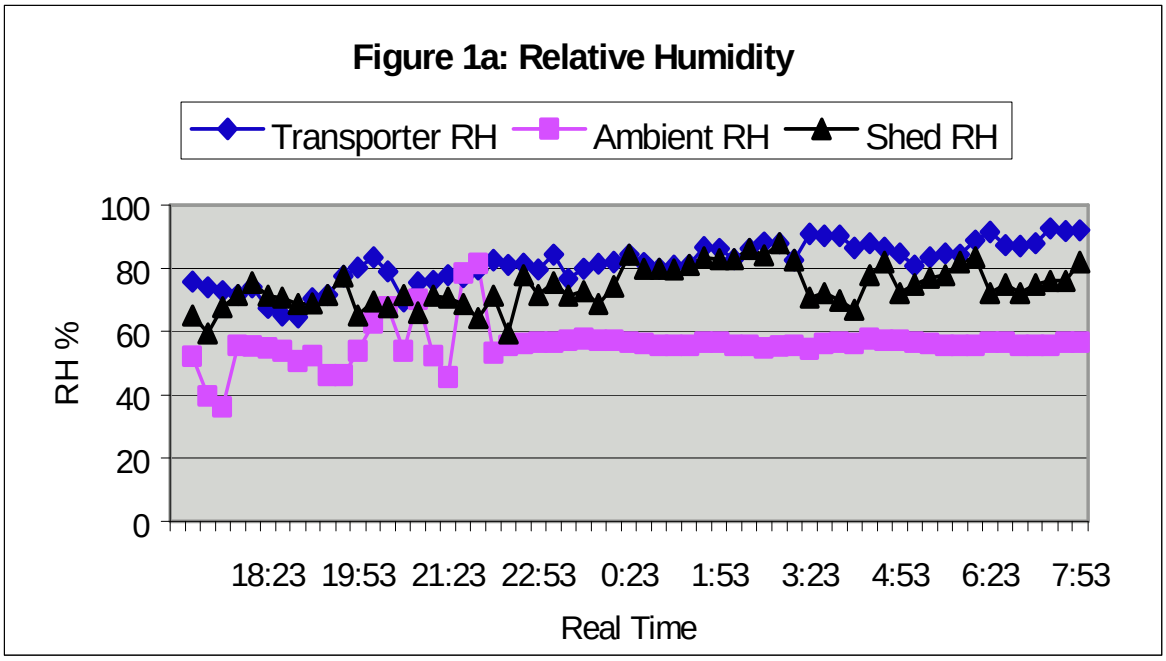


Figure 1a: Relative Humidity
Transporter RH
Ambient RH
Shed RH
100
80
60
40
20
0
RH %
18:23
19:53
21:23
22:53
0:23
1:53
3:23
4:53
6:23
7:53
Real Time

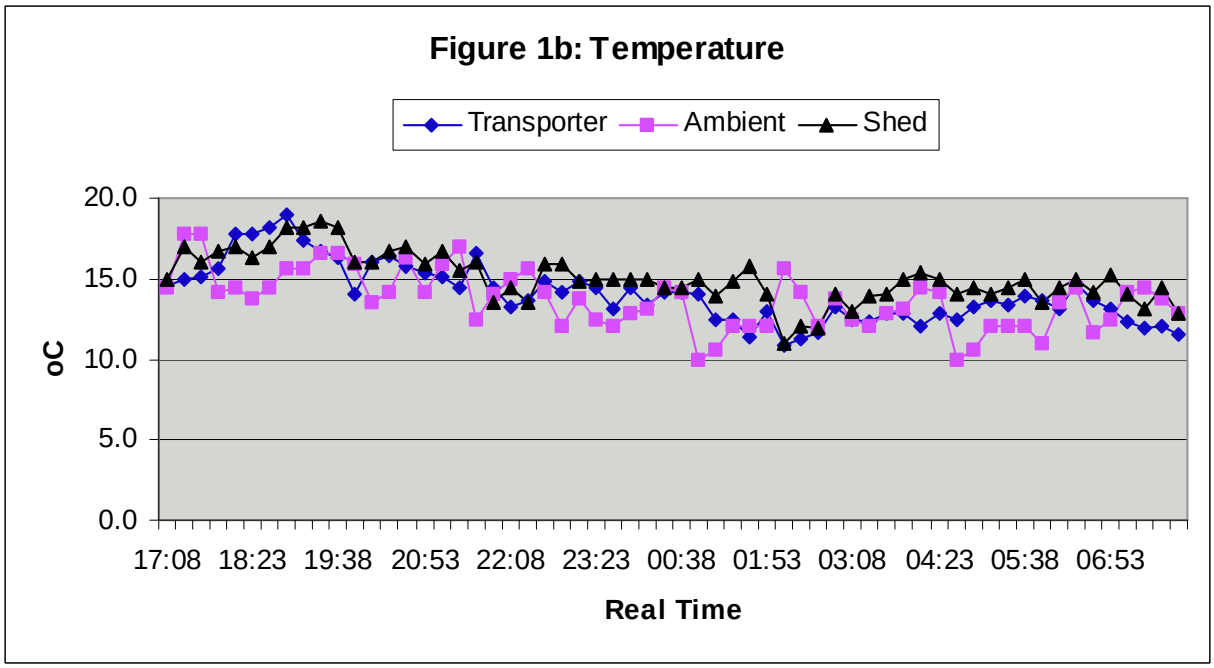


Figure 1b: Temperature
Transporter
Ambient
Shed
20.0
15.0
10.0
5.0
0.0
oC
17:08
18:23
19:38
20:53
22:08
23:23
00:38
01:53
03:08
04:23
05:38
06:53
Real Time

7. Statistical analysis

SAS/STAT® software was used to analyse the data. Pre-planned, matched pair t-test to detect changes over time were made using PROC MEANS, the null hypothesis being that the mean difference between selected time points was equal to zero. The PROC GLM repeated measures option was used to test the effects of treatment while controlling for time effects. Analysis was performed on the rank scores of variables that failed the test for normality.

8. Results and Discussion

8.1. Temperature, relative humidity during transport

Figure 1 summarises the environmental conditions (variation in temperature for ambient) on the lower deck of the transporter. The relative humidity (RH%) recorded in the transporter ranged from 64.4 (2:08am) – 90.7% (18:23pm) and the vapour density was 8.1 (2:08 am)– 13.2 (20:08pm) td^{o}C. In the shed the RH % ranged from 59.5 (22:23pm) – 88% (2:53am). The ambient relative humidity ranged from 45.6% (21:23pm) - 81.5% (21:53pm). The temperature (^{o}C) recorded in the transporter ranged from 10.0 (2:08am) – 18.9 (18:53pm). In the shed the temperature ranged from 11.0 (2:08am) – 18.5 (19:23pm). The ambient temperature ranged from 9.9 (0:53am) – 17.0 (21:23pm).
Carbon dioxide levels were recorded during transit and ranged from 334 (2:08am) – 1138 ppm (5:08am).

8.2. Rectal and Surface body temperature

There was no significant difference in rectal body temperature, pre- and post transport. However, all readings were significantly lower post-transport compared with pre-transport readings (Table 2a; Figure2).

12-hours post transport, the surface shoulder body temperature of the transported animals was significantly lower when compared with control animals (Table 2b).

The individual deep core body temperature for the transported and control animals were within the normal range and are illustrated in Figure 3.

Table 2a: Rectal body temperature in control and transported animals. Values are expressed as mean (^{o}C) ± SD with P values.

	Treatment	Day 1 rectal	Day 2 Rectal	Pair DIFF	Day 1 versus Day 2
1.27m^2	**Mean**	38.9	38.1	**0.0001**	
	SD	0.413	0.527		
0.85m^2	**Mean**	39.0	38.4	**0.0001**	
	SD	0.416	0.424		
control	**Mean**	38.6	38.5	**0.0454**	
	SD	0.327	0.321		

Tale 2b: Surface temperature in control and transported animals. Values are expressed as mean (^{o}C) ± SD with P values.

	Treatment	Day 2 Shoulder	Day 2 Rump	Day 2 Belly	Repeated	Sig. P =
1.27m^2	**Mean**	25.7	25.3	25.6	**Treat**	0.1214
	SD	1.433	2.011	1.866	**Body**	0.1837
					Treat * Body	0.0235
0.85m^2	**Mean**	24.7	24.8	25.5		
	SD	1.847	1.668	2.354		
control	**Mean**	27.2	25.7	25.2		
	SD	2	1.474	1.914		
	Treat Sig.	Control > 1.27m^2 and 0.85m^2	NS	NS		

NS non significant

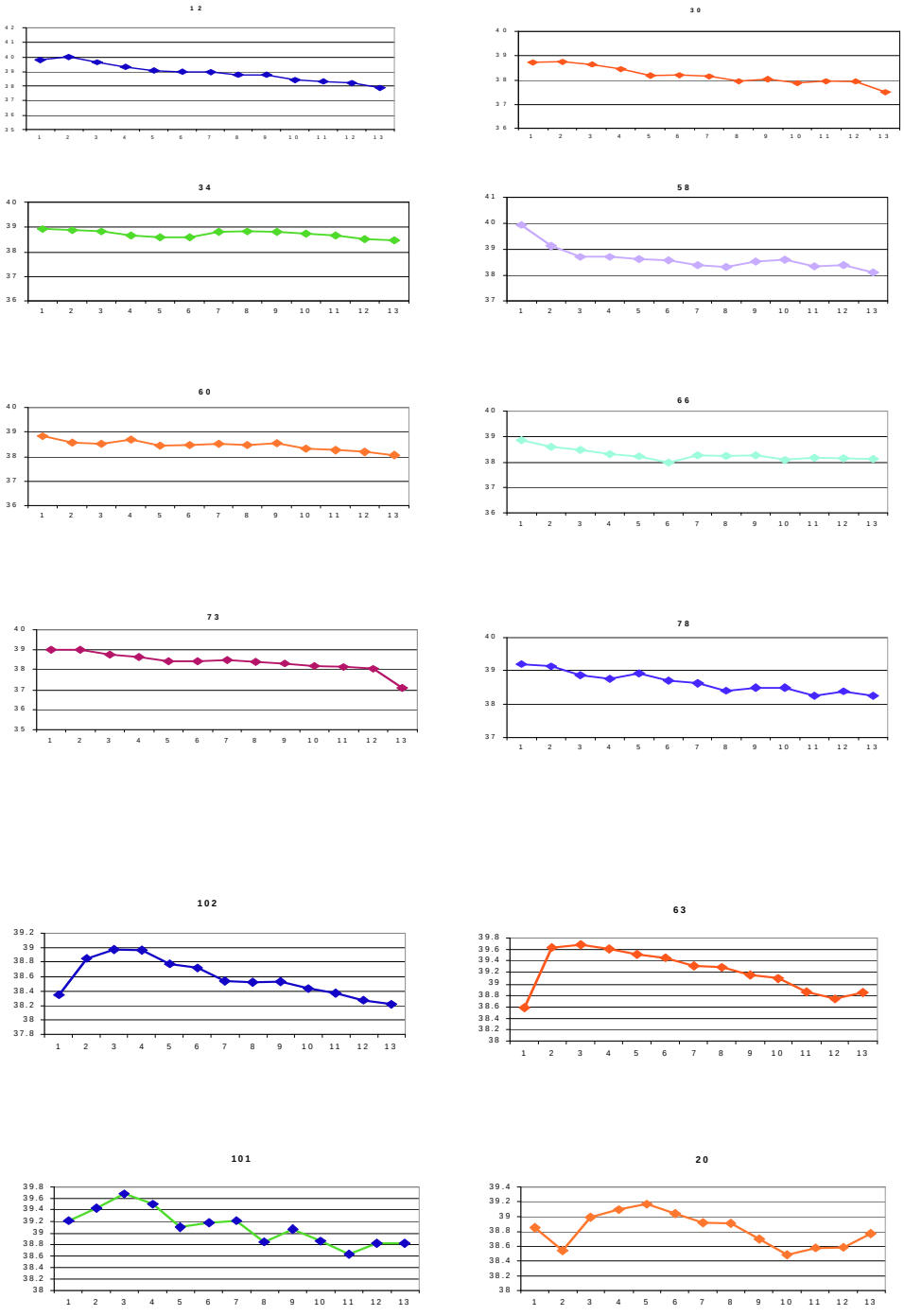


Figure: 3 Core body temperatures (^{o}C) for control and transported animals. There was no difference in core body temperature for animals transported at either of the two stocking densities;
(Control; Animal ID no's 20, 63, 101 and 102
0.85m^{2}; Animal ID no's 12, 30, 34, 58;
1.27m^{2}; Animal ID no's 60, 66, 73, 78).

8.3 Liveweight

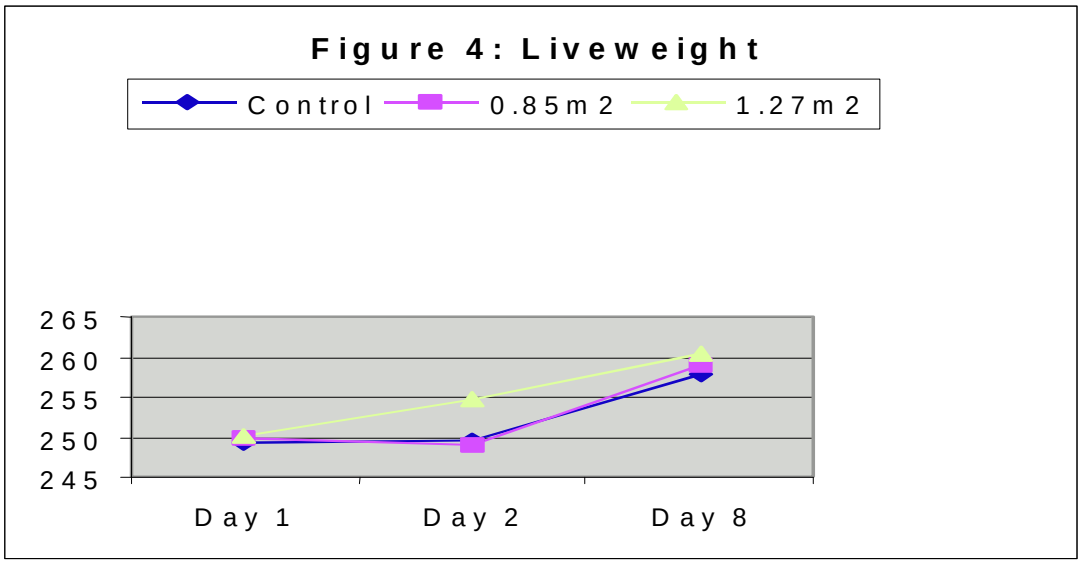
The changes in liveweight are shown in Table 3 and Figure 4.

There were no significant differences between the control and transported groups before the journey or on Days 2 and 8 after the journey. There was no significant difference in the rate of gain for either control or transported animals at either the $0.85m^2$ or the $1.27m^2$ stocking densities.

Table 3: Changes in liveweight in control and transported animals. Values are expressed as mean (kg) ± SD with P values.

	Treatment	Day 1	Day 2	Day 8	Repeated	Sig. P =
$1.27m^2$	**Mean**	**249.2**	**249.5**	**257.8**	**Treat**	0.9589
	SD	18.77	18.96	17.96	**Body**	0.0001
					Treat * Body	0.5448
$0.85m^2$	**Mean**	**249.8**	**249.1**	**258.9**	**Control > $1.27m^2$ and $0.85m^2$**	
	SD	20.72	20.25	24.64		
control	**Mean**	**250.2**	**254.6**	**260.4**		
	SD	43.38	43.26	41.07		
	Treat Sig.	NS	NS	NS		

NS non significant



9. Physiological variables

9.1 Albumin

There was no significant difference across the three treatments in albumin concentrations prior to transport (Table 4). Following transportation all transported groups had significantly higher albumin levels than the control animals. Post-transport, animals at the 0.85m^2 stocking density had significantly higher concentrations (P = 0.0322) than pre-transport. Control animals had significantly lower levels post-transport (P = 0.0001) compared with pre-transport baseline values.

9.2 Aspartate amino transferase (AST) and globulin (Glob)

There was no significant difference between treatments in AST or globulin concentrations prior to or after transport (Table 4). However values were significantly raised in all treatments (control and transport) at sample 2 when compared with sample 1 levels.

9.3 Betahydroxybutyrate (βHB)

There was no significant difference between treatments on βHB concentration prior to transport (Table 4). βHB concentrations were significantly decreased in the two transport groups post-transport and βHB concentrations were significantly higher in all groups relative to pre-transport values.

9.4 Globulin

Globulin concentrations were not significantly changed at any time during the experiment (Table 4). Protein measurement along with Albumin can indicate whether there has been an antibody response. (Total Protein - Albumin = Globulin) an increase in Gamma Globulins and a series of Acute Phase Proteins can result in an increase in the total Protein but this is usually somewhat offset by the reduction of Albumin in all Acute Phase situations. (Albumin is a "Reverse" Acute Phase Protein).

9.5 Glucose

There were no significant differences in glucose concentrations between treatments prior to transport (Table 4). Following transport, blood glucose concentrations were significantly higher in the transported animals at 0.85m^2 and 1.27m^2 compared with control values. Blood glucose

concentrations were significantly elevated at sample 2 compared with sample 1 values for all treatments.

9.6 Lymphocyte Numbers and % and Neutrophil Numbers and %

The % lymphocytes were reduced in the transported animals post-transport and there was no significant change in lymphocyte numbers (Table 4). The % of neutrophils and the number of neutrophils were significantly increased in the transported animals at $0.85m^2$ and $1.27m^2$ (Table 5).

9.7 Haematocrit (%) and red blood cell (RBC) numbers

The haematocrit values (Table 5) and RBC numbers (Table 8) were significantly higher in the transported bulls. However, haematocrit % for the animals at $1.27m^2$ were significantly higher than the control animals prior to transport. RBC numbers were similar prior to transport. However, haematocrit % was significantly higher in all treatment groups post-transport when the blood concentrations were compared with pre-transport values. RBC numbers were higher in the animals transported at a stocking density of $1.27m^2$ compared with control. It is also important to note that the haematocrit percentages and RBC numbers are within normal referenced ranges (Schalm, 1961).

9.8 Platelet numbers

Platelet numbers were not significantly changed at any time during the experiment (Table 5).

9.9 Protein and urea

Protein and urea concentrations were not significantly changed at any time during the experiment (Table 5).

10.0 White blood cells

White blood cell numbers were not significantly changed at any time during the experiment (Table 5).

10.1 Interferon-gamma production (IFN-γ)

There was no significant difference in the stimulated production of interferon-γ in response to concanavalin-A (Con-A) and keyhole limpet haemocyanin (KLH) between the three treatments prior to or after transport (Table 6).

10.2 Cortisol

Prior to transport, animals assigned to the $0.85m^2$ stocking density had higher cortisol concentrations than controls (Table 6). There were no significant differences in cortisol concentrations between treatments following transport.

10.3 Creatine phosphokinase (CK)

The activity of the enzyme creatine kinase was not altered by transportation at either the 0.85 or 1.27 m^2 stocking densities (Table 7). No change in the activity of CK, would indicate that the journey was not physically stressful.

10.4 Acute phase proteins (haptoglobin and fibrinogen)

Plasma haptoglobin concentrations were unchanged following transportation while plasma fibrinogen levels were significantly reduced in the two transported groups relative to controls (Table 7).

10.5 Haemoglobin

Haemoglobin levels for the animals at 1.27m^2 were significantly higher than the control animals prior to transport and after transport (Table 7).

10.6 Lactate

Lactate concentrations were not significantly changed at any time during the experiment (Table 7). Lactate is produced by anaerobic metabolism and is an indicator of muscle fatigue.

10.7 Lactate dehydrogenase

Lactate dehydrogenase (LDH) activity was unchanged following transportation while pre-transport values were significantly higher in the animals assigned to the 0.85 and 1.27 m^2 stocking density, prior to transport (Table 7).

10.8 Mean Cell Haemoglobin (MCH), Mean Cell Haemoglobin concentration (MCHC) , Mean cell volume (MCV).

MCH, MCHC and MCV were not significantly changed at any time during the experiment (Table 8).

10.9 Monocyte numbers, percentage (%) monocytes and platelet numbers.

There were no significant change in monocyte numbers, % monocytes and platelet numbers following transportation (Table 8).

10.10 Non-esterified fatty acids (NEFA)

Pre-transport NEFA concentrations were not significantly different. Following transport, animals transported at a stocking density of 1.27m^2 had significantly higher NEFA concentrations post transport compared with control values (Table 8). The concentrations for all treatment groups were significantly higher on Day 2 compared with Day 1 levels.

11. Conclusions

Bulls (250kg) undergoing 12-h transportation at stocking densities of 0.85 m^2 and 1.27m^2 showed physiological, haematological and immunological responses that were within normal referenced ranges. The responses were minimal and there was no significant change in either the immunological responses (interferon-γ production) or in plasma levels of the stress hormone, cortisol. Protein, globulin, urea and lactate concentrations, and white blood cell numbers were not significantly changed at any time during the experiment. The activities of the enzymes creatine kinase, aspartate aminotransferase and lactate dehydrogenase were not altered by transportation at either the 0.85 or 1.27 m^2 stocking densities. Following transportation all transported groups had significantly higher albumin levels than the control animals. There was no significant difference between treatments in beta-hydroxybutyrate concentrations (BHB) prior to transport. BHB concentrations were significantly decreased in all animals post-transport. Pre-transport non-esterified fatty acid (NEFA) concentrations were not significantly different. Following transport, animals transported at a stocked at 1.27m^2 had significantly higher NEFA concentrations compared with control values. There were no significant differences in glucose concentrations between treatments prior to transport. Post-transport, blood glucose concentrations were significantly higher in all transported animals compared with control values.

The % lymphocytes were reduced in the transported animals post-transport and there was no significant change in lymphocyte numbers. The percentage of neutrophils and the number of neutrophils were significantly increased in all transported animals. There were no significant change in monocyte numbers, % monocytes and platelet numbers following transportation. The haematocrit values and red blood cell (RBC) numbers were significantly higher in the transported bulls. However, haematocrit % for the animals at 1.27m^2 were significantly higher than the control animals prior to transport. RBC numbers were similar prior to transport while RBC numbers were higher in the animals transported at a stocking density of 1.27m^2 compared with control. Haemoglobin levels for the animals at 1.27m^2 were significantly higher than the control animals prior to and after transport. Plasma haptoglobin concentrations were unchanged following transportation while plasma fibrinogen levels were significantly reduced in the two transported groups. There was no significant difference in rectal body temperature, pre and post transport. There was no significant difference in the rate of gain for either control or transported animals at either the 0.85m^2 or the 1.27m^2 stocking densities.

There was no significant difference between the two stocking density treatments and thus there is no proof to support more loose stocking rate during transport.

Table 4: Effect of transport for 12 hours at 2 stocking densities ($0.85m^2$ and $1.27m^2$) on physiological, haematological an immunological variables taken before (Sample 1) and immediately after (Sample 2) the journey. Values are expressed as Mean ± SI with P values.

	Pre-transport			Post-transport		
		SAMPLE 1		**SAMPLE 2**		
Variable	**Treatment**	**MEAN**	**SD**	**MEAN**	**SD**	**PAIR DIFF**
ALBUMIN	$1.27m^2$	33.6	1.74	33.9	1.78	**0.273**
g/l	$0.85m^2$	33.0	1.70	33.7	1.29	**0.032**
	Control	32.8	1.75	31.1	1.55	**0.000**
	Sig.	**P = 0.4467**	**NS**	**P = 0.0001**		
				$1.27m^2$ and $0.85m^2$	> control	
Normal range 30.3 – 35.5 g/l (32.9 ± 1.3) (Kaneko, 1989)						
AST	$1.27m^2$	65.2	9.26	75.8	18.95	**0.029**
U/l	$0.85m^2$	61.9	9.63	74.6	13.97	**0.001**
	Control	62.9	8.00	73.1	11.78	**0.000**
	Sig.	**P = 0.6112**	**NS**	**P = 0.8813**	**NS**	
Normal range 78 - 132 U/l (105 ± 27) (Kaneko, 1989)						
BHB	$1.27m^2$	0.41	0.076	0.15	0.071	**0.000**
g/l	$0.85m^2$	0.43	0.109	0.17	0.054	**0.000**
	Control	0.39	0.084	0.25	0.068	**0.000**
	Sig.	**P = 0.5373**	**NS**	**P = 0.0006**		
				$1.27m^2$ and $0.85m^2$	< control	
Normal range (Kaneko, 1989)						
GLOBULIN	$1.27m^2$	40.0	5.76	44.0	5.16	**0.000**
g/l	$0.85m^2$	39.4	54.46	44.0	5.49	**0.000**
	Control	38.9	6.08	41.8	6.01	**0.000**
	Sig.	**P = 0.8729**	**NS**	**P = 0.4578**	**NS**	
Normal range 30.0 – 34.8 g/l (32.4 ± 2.4)						
GLUCOSE	$1.27m^2$	4.26	0.331	4.79	0.452	**0.012**
mmol/l	$0.85m^2$	4.16	0.283	5.01	0.405	**0.000**
	Control	4.16	0.528	4.21	0.279	**0.270**
	Sig.	**P = 0.7267**	**NS**	**P = 0.0001**		
				$1.27m^2$ and $0.85m^2$	> control	
Normal range 2.50 – 4.16 mmol/l (3.19 ± 0.38) (Kaneko, 1989)						
% Lymphocytes	$1.27m^2$	57.3	6.59	44.7	8.84	**0.000**
	$0.85m^2$	58.1	8.13	40.8	10.13	**0.000**
	Control	56.4	12.14	57.5	10.93	**0.582**
	Sig.	**P = 0.8794**	**NS**	**P = 0.0001**		
				$1.27m^2$ and $0.85m^2$	< control	
Normal range 45 – 75 (58) (Schalm, 1961)						
Lymphocyte No's	$1.27m^2$	6.0	1.28	5.2	1.61	**0.002**
	$0.85m^2$	6.3	1.02	5.0	1.10	**0.000**
	Control	5.9	1.04	5.9	1.35	**0.591**
	Sig.	**P = 0.6913**	**NS**	**P = 0.1786**	**NS**	
Normal range 1.8 – 9.00 4.64 (Schalm, 1961)						

NS non significant

Table 5: Effect of transport for 12 hours at 2 stocking densities ($0.85m^2$ and $1.27m^2$) on physiological, haematological and immunological variables taken before (Sample 1) and immediately after (Sample 2) the journey. Values are expressed as Mean ± SD with P values.

	Pre-transport			Post-transport		
		SAMPLE 1		SAMPLE 2		Pair
Variable	Treatment	MEAN	SD	MEAN	SD	Difference
% Neutrophils	$1.27m^2$	38.4	6.83	51.3	9.33	**0.0002**
	$0.85m^2$	38.2	8.00	55.6	10.05	**0.0001**
	Control	39.9	11.09	38.4	10.91	**0.4718**
	Sig.	**P = 0.8474**	**NS**	**P = 0.0001**		
				$1.27m^2$ and $0.85m^2$ > control		
Normal range 15-45 (28) (Schalm, 1961)						
Neutrophil No's	$1.27m^2$	4.0	1.05	6.0	1.66	**0.0029**
	$0.85m^2$	4.3	1.50	7.1	2.55	**0.0006**
	Control	4.5	2.06	4.1	1.89	**0.2007**
	Sig.	**P = 0.762**	**NS**	**P = 0.0008**		
				$1.27m^2$ and $0.85m^2$ > control		
Normal range 0.6 – 5.40 (2.24) (Schalm, 1961)						
(%)	$1.27m^2$	31.8	2.60	33.2	2.99	**0.0028**
Haematocrit	$0.85m^2$	30.9	2.60	32.5	3.46	**0.0025**
	Control	29.1	3.24	29.7	3.29	**0.0147**
	Sig.	**P = 0.0413**		**P = 0.012**		
		$1.27m^2$ > control		$1.27m^2$ > control		
Normal range 24-48 (35) (Schalm, 1961)						
Platelet No's	$1.27m^2$	959	246.2	975	235.2	**0.5076**
	$0.85m^2$	848	220.4	840	283.1	**0.8050**
	Control	862	224.6	828	266.9	**0.1800**
	Sig.	**P = 0.3872**	**NS**	**P = 0.279**	**NS**	
PROTEIN	$1.27m^2$	73.6	5.67	77.9	5.44	**0.0001**
g/l	$0.85m^2$	72.3	6.19	77.8	5.76	**0.0001**
	Control	71.6	6.91	73.0	6.97	**0.0330**
	Sig.	**P = 0.714**	**NS**	**P = 0.0501**	**NS**	
Normal range 67.4 – 74.6 g/l 71.0 ± 1.8 (Kaneko, 1989)						
UREA	$1.27m^2$	7.6	0.77	3.9	0.77	**0.0001**
mmol/l	$0.85m^2$	7.7	1.09	4.0	0.60	**0.0001**
	Control	7.8	1.18	4.4	0.90	**0.0001**
	Sig.	**P = 0.8569**	**NS**	**P = 0.2328**	**NS**	
Normal range 7.14 – 10.7 mmol/l (Kaneko, 1989)						
White blood cells	$1.27m^2$	10.5	2.02	11.7	2.53	**0.0796**
$X10^3$ µl	$0.85m^2$	10.9	2.13	12.5	2.64	**0.0283**
	Control	10.9	2.40	10.4	2.27	**0.1465**
	Sig.	**P = 0.8641**	**NS**	**P = 0.0593**	**NS**	
Normal range 4-12 (8.00) Schalm, 1961						

NS non significant

Table 6: Effect of transport for 12 hours at 2 stocking densities (0.85m^2 and 1.27m^2) on physiological, haematological and immunological variables taken before (Sample 1) and immediately after (Sample 2) the journey. Values are expressed as Median with minimum and maximum values. Non Parametric Kruskal-Wallis Test with P values.

		Pre-transport			Post-transport		
			SAMPLE 1		**SAMPLE 2**		**Pair**
	TEST	**TREAT**	**MEDIAN**	**MIN-MAX**	**MEDIAN**	**MIN-MAX**	**Difference**
	CONA	1.27m^2	0.168	0.019 - 0.831	0.133	0.021 - 0.627	**0.4548**
	Interferon-γ	0.85m^2	0.117	0 - 0.629	0.118	0.007 - 0.837	**0.6322**
	O.D.	Control	0.239	0.05 - 0.658	0.222	-0.012 - 0.635	**0.1152**
		Sig.	**P = 0.6526**	**NS**	**P = 0.978**	**NS**	
	KLH	1.27m^2	0.012	-0.022 - 0.092	-0.001	-0.051 - 0.074	**0.3054**
	Interferon-γ	0.85m^2	0.007	-0.022 - 0.077	0.010	-0.026 - 0.188	**0.6387**
	O.D.	Control	0.014	-0.015 - 0.179	0.022	-0.038 - 0.156	**0.9298**
		Sig.	**P = 0.6992**	**NS**	**P = 0.4507**	**NS**	
	Cortisol ng/ml	1.27m^2	8.004	-0.022 - 0.092	5.149	-0.051 - 0.074	**0.3757**
		0.85m^2	8.485	-0.022 - 0.077	7.784	-0.026 - 0.188	**0.7057**
		Control	5.217	-0.015 - 0.179	5.937	-0.038 - 0.156	**0.6322**
		Sig.	**P = 0.0403** 0.85m^2 > Control	**NS**	**P = 0.3118**	**NS**	

NS non significant

Table 7: Effect of transport for 12 hours at 2 stocking densities (0.85m^2 and 1.27m^2) on physiological, haematological and immunological variables taken before (Sample 1) and immediately after (Sample 2) the journey. Values are expressed as Median with minimum and maximum values. Non Parametric Kruskal-Wallis Test with P values.

	Pre-transport			Post-transport		
		SAMPLE 1		SAMPLE 2		Pair
TEST	TREAT	MEDIAN	MIN-MAX	MEDIAN	MIN-MAX	Difference
Creatine Kinase	1.27m^2	142.0	100 - 204	161.0	109 - 206	**0.2810**
	0.85m^2	156.5	95 - 226	160.5	125 - 986	**0.1473**
	Control	131.5	104 - 384	146.0	105 - 232	**0.9697**
	Sig.	**P = 0.5404**	**NS**	**P = 0.1585**	**NS**	
FIBRINOGEN mg/dl	1.27m^2	535.0	459 - 771	549.0	439 - 728	**0.3396**
	0.85m^2	510.0	449 - 1265	530.0	481 - 1546	**0.1135**
	Control	553.0	441 - 723	646.5	527 - 968	**0.0010**
	Sig.	**P = 0.9286**	**NS**	**P = 0.0366**		
HAPTOGLOBIN g Hb-binding capacity/l	1.27m^2	0.200	0.16 - 0.34	0.250	0.17 - 0.36	**0.9512**
	0.85m^2	0.215	0.16 - 3.02	0.215	0.17 - 2.45	**0.2942**
	Control	0.220	0.15 - 1.09	0.190	0.14 - 1.54	**0.2075**
	Sig.	**P = 0.9707**	**NS**	**P = 0.079**	**NS**	
Hb (gm. %)	1.27m^2	11.30	9.4 - 12.3	11.50	10 - 12.9	**0.0083**
	0.85m^2	10.80	9.1 - 12.4	11.30	9.6 - 13.3	**0.0023**
	Control	10.30	6.9 - 11.5	10.45	6.9 - 11.6	**0.0299**
	Sig.	**P = 0.0114**		**P = 0.0145**		
		1.27m^2 > control		1.27m^2 > control		
LACTATE mmol/l	1.27m^2	0.830	0.52 - 3.68	0.640	0.38 - 1.29	**0.0322**
	0.85m^2	1.075	0.62 - 1.97	0.770	0.55 - 1.93	**0.0146**
	Control	0.835	0.37 - 1.83	0.590	0.41 - 2.42	**0.0909**
	Sig.	**P = 0.1266**	**NS**	**P = 0.0737**	**NS**	
LDH U/L	1.27m^2	2204.0	1697 - 2878	1856.0	1608 - 2183	**0.0007**
	0.85m^2	2236.0	1467 - 3016	1680.0	1479 - 2576	**0.0008**
	Control	1709.5	1442 - 2432	1786.0	1424 - 2304	**0.4332**
	Sig.	**P = 0.0105**		**P = 0.59**	**NS**	
		1.27m^2 and 0.85m^2 > control				

NS non significant

Table 8: Effect of transport for 12 hours at 2 stocking densities ($0.85m^2$ and $1.27m^2$) on physiological, haematological and immunological variables taken before (Sample 1) and immediately after (Sample 2) the journey. Values are expressed as Median with minimum and maximum values. Non Parametric Kruskal-Wallis Test with P values.

	Pre-transport			Post-transport		
		SAMPLE 1		SAMPLE 2		Pair
TEST	TREAT	MEDIAN	MIN-MAX	MEDIAN	MIN-MAX	Difference
MCH	$1.27m^2$	12.10	10.5 - 17.8	12.00	10.5 - 17.4	**0.0195**
pg	$0.85m^2$	12.35	10.7 - 14.5	12.50	10.3 - 14.4	**0.8809**
	Control	12.50	11.2 - 14.7	12.40	11.3 - 14.9	**0.8950**
	Sig.	**P = 0.8219**		**P = 0.5622**		
MCHC	$1.27m^2$	35.20	34.1 - 35.8	34.90	33.9 - 35.4	**0.0315**
	$0.85m^2$	34.85	33.7 - 35.8	34.80	33.3 - 35.8	**0.2196**
g/dl	Control	35.00	33.3 - 35.7	34.85	33.3 - 35.5	**0.0708**
	Sig.	**P = 0.2989**		**P = 0.9784**		
Mean Cell Volume (MCV)	$1.27m^2$	34.70	30.6 - 50.8	34.80	30.2 - 51.3	**0.2808**
fl	$0.85m^2$	35.35	30.2 - 42.4	35.50	30.0 - 42.7	**0.2090**
	Control	36.10	33.3 - 42.2	36.05	33.5 - 42.3	**0.0559**
	Sig.	**P = 0.497**		**P = 0.5525**		
Normal range	40-60 fl					
MONOCYTE %	$1.27m^2$	2.5	1 - 6	2.0	1 - 5	**0.5938**
	$0.85m^2$	2.0	1 - 4	2.5	1 - 5	**0.2554**
	Control	2.0	0 - 5	2.0	1 - 6	**0.2451**
	Sig.	**P = 0.4011**		**P = 0.9105**		
MONOCYTE No	$1.27m^2$	0.240	0.1 - 0.91	0.310	0.09 - 0.55	**0.7485**
	$0.85m^2$	0.215	0.12 - 0.44	0.330	0.09 - 0.70	**0.0674**
10^9/l	Control	0.215	0 - 0.73	0.275	0.11 - 0.64	**0.6580**
	Sig.	**P = 0.6303**		**P = 0.3275**		
NEFA	$1.27m^2$	0.130	0.11 - 0.17	0.290	0.19 - 0.56	**0.0002**
µmol/l	$0.85m^2$	0.135	0.09 - 0.39	0.295	0.21 - 0.42	**0.0001**
	Control	0.135	0.11 - 0.16	0.150	0.08 - 0.22	**0.0599**
	Sig.	**P = 0.8156**		**P = 0.0001**		
				$1.27m^2$ and $0.85m^2$ > control		
Red Blood cell No's	$1.27m^2$	9.320	5.59 - 10.6	9.600	5.78 - 11.8	**0.0032**
X 10^6	$0.85m^2$	8.525	6.25 - 10.5	9.050	6.68 - 11.9	**0.0034**
	Control	8.360	4.66 - 9.74	8.530	4.65 - 9.58	**0.0220**
	Sig.	**P = 0.0915**		**P = 0.042**		
				$1.27m^2$ > control		

NS non significant

References

Eldridge, C.A., Winfield, C.G., and D.J. Cahill, (1988). Responses of cattle to different space allowances, pen sizes and road conditions during transport. Australian Journal of Experimental Agriculture 28:155-159.

Kaneko, J.J., (1989). Clinical biochemistry of domestic animals. 4th Edition, Academic Press Inc.mSan Diego, pp 886-891.

Lambooy, E., and B. Hulsegge. (1988). Long-distance transport of pregnant heifers by truck. Appl. Anim. Behav. Sci. 20:249.

Randall, J. M. (1993). Environmental parameters necessary to define comfort for pigs, cattle and sheep in livestock transporters. *Animal Production* **57**: 2, 299-307.

SAS/STAT ® Software Version 6.1 of the SAS System for windows. Copyright © 1989-1996 SAS Institute Inc. SAS and all other SAS Institute Inc. product or services names are registered trademarks or trademarks of SAS Institute Inc. Cary, NC, USA.

Schulm, O.W. (1961). Ballieree Tindall and Cox Covent Garden london, UK pp 130-131.

Tarrant, P.V., and T. Grandin. (2000). Cattle Tansport ; in T. Grandin (Editor) Livestock Handling and Transport (2nd Edition), CAB International, U.K.

Tarrant, P.V., Kelly, F.J., and D. Harrington, D. (1988). The effect of stocking density during 4 hour transport to slaughter, on behavior, blood constituents and carcass bruising in Friesian steers. Meat Science 24:209-222

Tarrant, P.V., Kenny, F.J., Harrington, D., and M. Murphy. (1992). Long distance transportation of steers to slaughter: effect of stocking density on physiology, behavior and carcass quality. Livestock Production Science 30:223-238

Acknowledgments

The authors gratefully acknowledge the technical assistance of: Francis Collier, Martin Donlon, Sandeep Gupta, Joe Larkin, Paddy Mallon, Ann Marley, Mary Munnelly, Joe Munroe, Liam Moore, Michael Nolan, Julianne Price, Simon Perry, Dan Prendiville, Many thanks are due to: the farm foreman, Gerry Santry, farm staff – Gabriel Costelloe, Joe Gill, 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.