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Effects of pre-transport fasting on the physiological responses of young cattle to 8-hour road transport

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The effects of fasting animals for 8 h prior to an 8-h road journey and their ability to cope with the stress of transport were investigated. The treatments were: 1) fasted and then transported (n=20); 2) non-fasted and transported (n=18); 3) non-fasted at grass (n=18); 4) fasted then fasted (n=18), and 5) non-fasted then fasted (n=18). There was no significant difference in rectal body temperature, pre- or post-transport, or live weight among treatments on days 0 (pre-transport), 1, 4 or 10 (post-transport). The ambient relative humidity and temperature of the outside environment ranged from 82.8 to 99.8% and 9.9 to 14.5 °C, respectively. Holstein × Friesian bulls (230 kg) undergoing an 8-h transportation at stocking densities of 0.82 m²/animal showed physiological and haematological responses that were within normal referenced ranges. Animals that were fasted for 8 h and transported lost 9.4% of live weight while non-fasted transported animals lost 7.2%. The control non-fasted animals remaining at grass gained 2% of live weight. Animals that were fasted continuously but not transported and the initially non-fasted control animals that were subsequently fasted for 9 h lost 6.1% and 6.2% of live weight, respectively. There was no significant change in concentrations of globulin, glucose, urea, haemoglobin or fibrinogen, or in haematocrit percentage before or after transport. Transport reduced lymphocyte percentage (P < 0.001) and increased neutrophil percentage (P < 0.001) in the fasted and non-fasted animals. Following transport, protein concentration was greater ($P \le 0.001$) in the fasted and transported animals than in the non-fasted animals at grass and haptoglobin concentrations were higher ($P \le 0.001$) in the fasted plus transported animals than the controls at grass. In conclusion, from

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the physiological and haematological measurements, an 8-h journey time, even without access to feed for 8 h prior to transport did not appear to impact negatively on animal welfare.

Keywords: Animal welfare; immunology; physiology; transport

Introduction

The transport of livestock can have major implications for their welfare, and there is strong public interest and scientific endeavour aimed at ensuring that the welfare of transported animals is optimal. Current EU Directives on animal welfare during transportation require cattle to be fed every 24 h (CEC, 1991), and there is expert opinion that the feeding interval during transport should be reduced to 12 h (SCAHAW, 2002). Transport conditions have the potential to alter the physiological responses of young bulls to the psychological or physical stress of transport. One of the concerns regarding cattle transport is that the handling and marketing of animals prior to a journey may lengthen the period of feed withdrawal (Warriss, 1990; Warriss and Brown, 1994). These periods of premovement/transport fasting can be difficult to control, yet there is ample evidence that extended periods of feed withdrawal can result in live-weight loss and reductions in meat quality in animals that are transported to slaughter (Bass and Duganzich, 1980; Smith et al., 1982). Furthermore, feed withdrawal can impact on animal welfare (Warriss, 1992), through hunger and metabolic stress. Changes in plasma concentrations of metabolic markers such as lactate, beta-hydroxybutyrate and urea have been used to measure the metabolic impacts of transport on cattle (Warriss et al., 1995). Alterations in immune function are an additional potential impact of transport that are particularly relevant to younger animals (Blecha, Boyles and Reilly, 1984; Phillips et al., 1989), and illness in young cattle following road haulage

is not uncommon and is due to transport or mixing of groups from many sources.

Although recommendations in Europe are to minimise the durations of periods without feed for transported cattle, conversely, in some countries, such as New Zealand, there are recommendations that livestock should be fasted for 4 to 6 h before a journey (Anonymous, 1994), with a view to reducing faecal output during transport, and thereby facilitating a more comfortable journey.

Given the practical difficulties in ensuring that all marketed cattle have only a minimal feed withdrawal period before subsequent transport, the objective of this study was to investigate the effects of an 8-h fast, prior to an 8-h transport journey, on the physiological and haematological responses of young cattle.

Materials and methods

The study utilised 92 (8-month-old) Holstein × Friesian bull calves (mean weight (s.d.) 229.6 (32.9) kg) that were managed at pasture. In July 2002, the animals were randomly assigned by live weight to one of five treatments: 1) fasted and transported (FT) (n=20); 2) non-fasted and transported (NFT) (n=18); 3) non-fasted at grass (NFG) (n=18); 4) fasted then fasted (FF) (n=18), and 5) non-fasted then fasted (NFF) (n=18). The groups that were subjected to the 8-h pre-transport fasting period (FT and FF) were removed from the grazing area to a sacrifice paddock with no access to grass but with access to water. The animals that were not transported but were fasted during the transport period (FF and NFF) were also held in adjacent sacrifice paddocks for 9 h with no access to grass but with access to water. The transported bulls were carried on the lower deck of an air-sprung articulated transporter (total floor area 30.96 m²) which was equally divided into 4 fan-ventilated pens at a stocking density of 0.82 m²/animal. The journey commenced at 2145, and consisted of 4 h of travel, followed by a 1-h rest period, followed by a further 4 h of travel to the point of origin. The total journey (474 km) involved a combination of road surfaces ranging from motorways, secondary roads to small country lanes.

All the animals were blood sampled by jugular venipuncture immediately before the transport period (day 0; pre-transport), and again at the end of the transport period (day 1; post-transport).

Body temperature

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 on days 2, 3, 5, 7, 9, 11 and 38 of the study. The surface body temperature (shoulder, rump, belly) of all animals was recorded using a hand held laser device (Raytek MX series 16 point laser, Raytek, P.O. Box, 120, Milton Keynes, MK1 1ZU, UK) on completion of the 8-h journey. The ambient temperature and relative humidity (RH) during transport and in the housing environments were recorded continuously using TinyTalk dataloggers (Gemini Data loggers, UK). Environmental measurements on the transporter, including RH and temperature, were recorded continuously during transit using QRae computerised logging systems (Shawcity Ltd., UK).

Live weight

All animals were weighed immediately before the transport period (day 0; pretransport), and again at the end of the transport period (day 1; post-transport) and on days 4 and 10.

Physiological, haematological and immunological variables

Blood samples, collected by jugular venipuncture and placed into heparinised tubes, were centrifuged and the plasma separated for subsequent analysis of: glucose, β -hydroxybutyrate (β HB), urea, total protein, albumin globulin, and the acute phase proteins (fibrinogen and haptoglobin). Haematological variables, including red blood cell number (RBC), mean corpuscular haemoglobin (Hb) concentration (MCHC), haematocrit (packed cell volume), mean cell volume (MCV), white blood cell (WBC) number, percentage lymphocytes and neutrophils, were determined for unclotted (K₂-EDTA) whole blood samples using an electronic particle haematology analyser (Celltac, MEK-6108K, Nihon-Kohdon, Tokyo, Japan). Thin blood smears were placed on greasefree glass slides (Gold star micro slides, Chance Propper Ltd., UK) and were prepared for WBC differential population count. The smears were air dried and stained using the haematology three-step stain for differentiation of morphological cell types (Accralab, Fisher Scientific Company, L.L.C., 8365 Valley Pike, Middleton, VA 22645-0307, USA). Plasma haptoglobin concentration was measured by determining the haemoglobin-binding capacity using a biochemical autoanalyser (Skinner, Brown and Roberts, 1994). Fibrinogen concentrations were measured using a commercial biochemical assay kit (Boehringer Mannheim, Germany). All other physiological measurements were made using Randox assay procedures.

Statistical analysis

Data were analysed using the various procedures of SAS/STAT (SAS, 1996).

Physiological and haematological measurements for globulin, glucose, protein, urea, MCHC, lymphocyte percentage, lymphocyte number, neutrophil percentage, neutrophil number, haematocrit percentage, and WBC were analysed by a one-way ANOVA (Proc GLM), using a means statement with a Tukey option to evaluate treatment differences. A matched-pair t-test was used to evaluate differences pre- and post-transport for each treatment. Live weight data and rectal and surface temperature measurements were analysed using the repeated measures procedure in Proc GLM. Physiological and haematological measurements for albumin, fibrinogen, haptoglobin and β HB, were not normally distributed. They were analysed by Proc GLM, using ranked data in a Kruskal-Wallis test with the Tukey option to evaluate treatment differences. The Wilcoxon signed rank test was used to evaluate differences pre- and post-transport for each treatment (Snedecor and Cochran, 1989).

Results

Environmental conditions during transport The relative humidity recorded in the transporter ranged from 74.5 to 94.4%, while the temperature ranged from 11.0 to 15.4 °C. The percentage carbon dioxide, wind velocity and vapour density recorded during transport were 0.06 to 0.11%; 0.26 to 0.50 m/s and 8.6 to 13.8 g/m³, respectively. The ambient relative humidity and temperature ranged from 82.8 to 99.8% and 9.9 to 14.5 °C, respectively.

Live weight

The changes in live weight are shown in Table 1. There were no significant differences in live weight among treatments on days 0 (pre-transport), 1 (post-transport), 4 or 10. Animals in the FT lost 9.4% live weight during the 8-h journey, while NFT

Table 1. Mean live weight (kg) of fasted andnon-fasted control and transported animalson days 0, 1, 4 and 10

Treatment ¹	Day ²				s.e.
	0	1	4	10	
FT	227.5 ^a	206.1 ^b	229.2ª	239.4 ^b	5.25
NFT	231.2ª	214.6 ^b	230.7 ^a	242.9 ^b	8.34
NFG	226.1ª	230.1 ^b	226.3ª	239.6 ^b	9.71
FF	230.7 ^a	216.7 ^b	233.6 ^b	242.8 ^b	7.06
NFF	233.2ª	218.8 ^b	228.8 ^a	240.9 ^b	7.55

 ${}^{1}\text{FT}$ = fasted and transported, NFT = non-fasted and transported, NFG = non-fasted at grass, FF = fasted then fasted, NFF = non-fasted then fasted. ${}^{2}\text{Day }0$ = pre-transport.

^{a,b}Means, within rows, without a superscript in common differ significantly $P \le 0.05$.

animals lost 7.2%. The animals remaining at grass (NFG) gained 2% over the same period. The FF and NFF animals lost 6.1% and 6.2%, respectively, between day 0 and day 1. By day 10 of the study, the live weights of all the animals were higher (P \leq 0.001) than their pre-transport values.

Body temperature

There were no differences (P > 0.05) in rectal temperature among treatments on day 0 (pre-transport) (Table 2). The repeated-measures analysis of variance showed no significant difference in rectal temperature between the five treatments (P = 0.10).

Table 2. Mean rectal temperature (°C) of fasted and non-fasted control and transported animals on days 0, 1 and 10

Treatment ¹		Day ²		s.e.
	0	1	10	-
FT	38.6	38.4	37.1	0.08
NFT	38.8	38.5	39.0	0.08
NFG	38.8	38.5	39.0	0.25
FF	38.5	38.5	39.1	0.09
NFF	38.9	38.6	39.1	0.10

 ${}^{1}\text{FT}$ = fasted and transported, NFT = non-fasted and transported, NFG = non-fasted at grass, FF = fasted then fasted, NFF = non-fasted then fasted. ${}^{2}\text{Day } 0$ = pre-transport.

Physiological, haematological and immunological variables

Globulin: There was no significant difference (P > 0.05) in globulin concentrations among treatments prior to or after transport (Table 3). Comparing post and pre transport for each treatment, globulin concentrations were significantly higher in the FT, NFT and NFF treatments post transport.

Glucose: There was no significant difference in glucose concentrations among treatments prior to transport. Plasma glucose concentrations were higher ($P \le 0.001$) post transport in FT and NFT compared to the other treatments (Table 3). Comparing post and pre transport for each treatment, glucose concentrations were higher ($P \le 0.001$) in the FT and NFT and lower in the FF treatments post transport.

Protein: Protein concentrations in plasma were lower ($P \le 0.001$) in the NFT and NFF treatments compared to the other treatments prior to transport. Following transport, protein concentrations were higher ($P \le 0.001$) in the FT treatment than the NFG treatment (Table 3). Comparing post and pre transport for each treatment, protein concentrations were higher ($P \le 0.001$) in the FT, NFT, FF and NFF post-transport.

Urea: Urea concentrations were not significantly different among treatments. Comparing post and pre transport for each treatment, urea concentrations were lower ($P \le 0.001$) in the NFT treatment post-transport.

Betahydroxybutyrate: β HB concentrations were different (P \leq 0.001) among treatments pre-transport with FT animals having lower concentration and NFT animals having higher concentration compared with NFG, FF and NFF treatments. Following transport, FT, FF and NFF treatments had lower (P \leq 0.001) β HB concentrations than the NFG treatment (Table 3). Comparing post and pre transport for each treatment, β HB concentrations were lower (P \leq 0.001) in the NFT and NFF treatments and higher (P \leq 0.001) in the FT and FF treatments post transport.

Albumin: Albumin concentrations were lower (P \leq 0.001) in the NFF before transport. Following transport, albumin concentrations were higher (P \leq 0.001) in FT than in NFG, FF, and NFF and the NFT treatment had lower (P \leq 0.001) albumin concentrations than the FT treatment (Table 3). Comparing post and pre transport for each treatment, albumin concentrations were higher (P \leq 0.001) in the FT, NFT and NFF treatments post-transport.

Plasma constituent	Treatment ¹					
	FT	NFT	NFG	FF	NFF	
Globulin (g/L)	43.5 ^a (0.95)	$42.8^{a}(1.04)$	41.6 (1.15)	43.7 (0.88)	42.5 ^a (1.19)	
Glucose (mmol/L)	5.0 ^a (0.07)	$5.0^{a}(0.12)$	4.2 (0.04)	4.0 ^a (0.07)	4.1 (0.09)	
Protein (g/L)	81.3 ^a (0.92)	79.5 ^a (1.05)	76.8 (1.17)	79.7 ^a (1.06)	77.5 ^a (1.02)	
Urea (mmol/L)	5.1 ^a (0.15)	$4.7^{a}(0.18)$	5.4 (0.27)	5.4 (0.14)	5.4 (0.18)	
Beta hydroxy butyrate (g/L)	$0.26^{a}(0.02)$	$0.31^{a}(0.03)$	0.38 (0.02)	$0.27^{a}(0.02)$	$0.27^{a}(0.02)$	
Albumin (g/L)	37.6 ^a (0.03)	36.7 ^a (0.26)	35.4 (0.45)	35.8 (0.34)	35.5 (0.46)	
Fibrinogen (mg/dL)	656.5 ^a (15.5)	659.4ª (32.4)	637.1 (26.5)	609.6 (25.8)	640.9 ^a (22.0)	

Table 3. Treatment effects on mean (s.e.) values for blood plasma constituents post-transport

 1 FT = fasted and transported, NFT = non-fasted and transported, NFG = non-fasted at grass, FF = fasted then fasted, NFF = non-fasted then fasted.

^aMean value differs significantly ($P \le 0.05$) from pre-transport value.

Fibrinogen: Fibrinogen concentrations were not significantly different among treatments prior to or after transport (Table 3). Comparing post and pre transport for each treatment, fibrinogen concentrations were lower ($P \le 0.001$) in the FT and NFG treatments post transport.

Mean corpuscular haemoglobin concentration: There was no significant difference in mean corpuscular haemoglobin concentration among treatments prior to or after transport (Table 4). MCHC concentrations were lower in the FF treatment post transport compared with pre transport.

White blood cells: WBC numbers were not significantly different among treatments pre transport. Post transport, the WBC numbers were higher ($P \le 0.001$) in the FT treatment than the NFG, FF, and NFF treatments (Table 4). Comparing post and pre transport for each treatment, WBC numbers were higher ($P \le 0.001$) in the FT and NFT and lower ($P \le 0.001$) in the NFG, FF and NFF treatments.

Lymphocytes: There was no significant difference in lymphocyte percentage

between treatments prior to transport. The percentage of lymphocytes was reduced in the transported animals (FT and NFT) post transport compared to the other treatments (Table 4). Comparing post and pre transport for each treatment, the lymphocyte percentage was lower ($P \le 0.001$) in the FT, NFT and FF treatments post transport.

Lymphocyte numbers were different (P \leq 0.001) among treatments pre transport with NFT and NFF having higher numbers than the FT treatment. Following transport there was no significant difference in lymphocyte numbers among treatments (Table 4). Comparing post and pre transport for each treatment, lymphocyte numbers were lower (P \leq 0.001) in the FT, NFT, NFG and NFF treatments post transport.

Neutrophils: The percentage of neutrophils and the number of neutrophils were increased ($P \le 0.001$) in the FT and NFT animals, while NFG, FF and NFF had lower ($P \le 0.001$) neutrophil numbers after transport compared to the other treatments (Table 4). Comparing post and pre transport for each treatment, the percentage of

Haematological variable	Treatment ¹					
	FT	NFT	NFG	FF	NFF	
Mean corpuscular haemoglobin concentration (g/dL)	11.4 (0.15)	11.7 ^a (0.15)	11.5 (0.16)	11.5 ^a (0.17)	11.6 (0.20)	
Number of white blood cells ³ ($\times 10^{-3}$)	15.5 ^a (0.57)	15.1 ^a (0.58)	12.9 ^a (0.76)	10.7 ^a (0.37)	$11.8^{a} (0.50)$	
Number of lymphocytes ³ ($\times 10^{-3}$)	$6.3^{a}(0.25)$	6.4 ^a (0.31)	7.1 ^a (0.32)	5.6 (0.29)	$6.8^{a}(0.35)$	
Percent lymphocytes ⁴ (%)	34.5 ^a (1.76)	36.4 ^a (2.70)	48.9 (1.49)	50.4 ^a (1.85)	51.3 (2.27)	
Number of neutrophils ³ ($\times 10^{-3}$)	7.1 ^a (0.56)	$6.7^{a}(0.61)$	7.3 ^a (0.52)	$6.9^{a}(0.50)$	$6.6^{a}(0.55)$	
Percent neutrophils ⁴ (%)	63.8 ^a (1.73)	61.1 ^a (2.82)	48.3 (1.58)	46.6 ^a (1.88)	46.0 ^a (2.45)	
Number of red blood cells ³ (\times 10 ⁻⁶)	$10.3^{a}(0.15)$	$9.8^{a}(0.19)$	9.8 (0.15)	10.0 ^a 90.18)	9.73 (0.26)	
Haptoglobin ²	0.29 (0.04)	0.19 (0.02)	$0.25^{a}(0.10)$	0.19 (0.02)	$0.20^{a}(0.03)$	
Haematocrit (%)	34.6 ^a (0.43)	34.0 ^a (0.56)	33.1ª (0.49)	34.3 ^a (0.53)	33.4 (0.67)	

Table 4. Treatment effects on mean (s.e.) values for haematological variables post-transport

 1 FT = fasted and transported, NFT = non-fasted and transported, NFG = non-fasted at grass, FF = fasted then fasted, NFF = non-fasted then fasted.

²Measured as haemoglobin binding capacity.

⁴As percentage of total white blood cells.

^aMeans differ significantly from pre-transport values $P \le 0.05$.

³Per microlitre.

neutrophils was increased ($P \le 0.001$) in the FT and NFT treatments and decreased in the FF treatment post-transport. Comparing post and pre transport for each treatment, the number of neutrophils was increased ($P \le 0.001$) in all treatments.

Red blood cells: RBC numbers were not significantly different among treatments pre or post transport (Table 4). Comparing post and pre transport for each treatment, median RBC numbers were significantly higher in the FT, NFT and FF treatments post transport.

Haptoglobin: Haptoglobin concentration did not significantly differ among treatments pre transport. Post transport, haptoglobin concentrations were higher ($P \le 0.001$) in the FT than the NFG treatment (Table 4). Comparing post and pre transport for each treatment, haptoglobin concentrations were lower ($P \le 0.001$) in the NFG treatment post transport.

Haematocrit: There was no significant difference in haematocrit percentage among treatments before or after transport (Table 4). Comparing post and pre transport for each treatment, haematocrit percentage was higher ($P \le 0.001$) in the FT, NFT, FF treatments and lower in the NFG treatment post transport.

Discussion

The physiological and haematological measurements indicate that an 8-h journey time, even without access to feed for 8 h prior to transport, did not impact negatively on animal welfare. The increase in glucose concentrations in FT and NFT treatments following transport could have been due to catecholamine-stimulated glycogenolysis. An increase in neutrophil and decrease in lymphocyte numbers, increases in white blood cell count and glucocorticoid release can be indicative of disease, inflammation and many other types of stress. Immune

suppression in calves has been associated with increased plasma cortisol and acute phase protein (haptoglobin) production (Blecha and Baker, 1986; Murata and Miyamoto, 1993). Haptoglobin production by calf liver parenchymal cells is inducible with glucocorticoids *in vitro* (Higuchi *et al.*, 1994). It is interesting to note that haptoglobin concentrations were significantly higher in the fasted and transported bulls compared with the non-fasted controls at grass and suggests that the immune system may have been compromised.

The effects of previous experience on an animal's fear response may provide one explanation for the often variable results in handling and transport studies. For example, extensively raised animals may experience more stress during loading and unloading for transport than more intensively reared animals. It is well established that animals that are accustomed to frequent handling and close contact with people are usually less stressed by restraint and handling. Binstead (1977), Fordyce et al. (1985), and Fordyce (1987) report that training weanling heifer calves produced calmer adult animals that were easier to handle. The influence of the type of animal transported and their responses to transport-induced stress response was investigated by Tennessen, Price and Berg (1984). In their studies, they measured heart rate, serum cortisol concentration and body temperature over a series of seven journeys in bulls (513 kg live weight) and steers (473 kg live weight). They reported that the cortisol response to transport and increase in body temperature was greater for steers than bulls over 2-h journeys, and Tennessen et al. (1984) reported similar and minimal overall responses for both sets of animals. It was reported by Grandin (1997) that the often variable results from animal handling and transport studies may be related to the effects of previous experience on an animal's fear response. Mormede, Bluthe and Dantzer (1983) showed no consistent cortisol response to transport in calves transported by road for 3 h, while calves that were held without feed or water overnight, and transported for 8 h showed signs of dehydration by the end of the journey, and were hypoglycaemic until 1 week after transport. Transportation of cattle has also been associated with the development of electrolyte and acid-base imbalances, particularly in extended journeys where the total fasting time exceeds 2 days (Schaefer et al., 1988), and with the suppression of reproductive function (Nanda, Ward and Dobson, 1989). The physiological responses of cattle to transport have been documented by Leach (1981) while Shaw and Tume (1992) have reported the effects of pre-slaughter handling on the plasma levels of blood constituents. Tarrant et al. (1992) reported increased RBC, packed cell volume and Hb following transportation of steers while Lambooy and Hulsegge (1988) reported increased haematocrit in pregnant heifers. By contrast Blecha et al. (1984) reported no changes in the packed cell volume in shipped calves. In the present study, albumin concentration increased in the fasted and transported bulls following transport with no change in haematocrit percentage. Transport stress has been reported to cause dehydration and may manifest itself as a hyperproteinemia (Atkinson, 1992; Schaefer et al., 1992). Serum proteins, especially albumin, act as weak acids in plasma (Radostits, Blood and Gay, 1994). The role of proteins in acid-base balance has practical importance. Thus, hypoproteinaemia and hyperproteinaemia by themselves cause metabolic alkalosis and acidosis, respectively. The changes in albumin levels seen post-transport are more likely the result of a respiratory and/or metabolic compensation for a mild metabolic acidosis secondary to water loss and feed deprivation.

In the present study, the percentage of neutrophils and the number of neutrophils were significantly increased in the FT and NFT animals after transport. The changes in the composition of the blood cell variables reflect the physiological or pathophysiological response of the body to the stress of fasting and/or transportation. The neutrophilia and lymphopenia following transportation observed in this study are in agreement with previously reported findings following a variety of stressors, including transportation stress (Blecha et al., 1984; Murata, Taklahasi and Matsumoto, 1987; Tarrant et al., 1992). Neutrophilia and lymphopenia is a common finding in stressed animals and is associated with changes in the WBC trafficking and release from the bone marrow by the elevated concentrations of the glucocorticoids (Dunn, 1989).

In conclusion, the results of this study, show that from the physiological and haematological measurements, an 8-h journey time, even without access to feed for 8 h prior to transport did not appear to impact negatively on animal welfare.

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