**End of Project Report**

# EFFECTS OF PRE-JOURNEY FASTING ON THE PHYSIOLOGICAL RESPONSES OF YOUNG CATTLE TO 8-HOUR ROAD TRANSPORT

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## 1. SUMMARY

The present study evaluated the effects of fasting animals for 8 hours prior to an 8-hour road journey and their ability to cope with the stress of transport. There was no significant difference in rectal body temperature, pre and post transport and there were no significant differences in liveweight among treatments on days 0 (pre-transport), 1, 4 and 10 (post-transport). Bulls (230kg) undergoing an 8-h transportation at stocking densities of 0.82 $m^2$ /animal showed physiological and haematological responses that were within normal referenced ranges. Animals that were fasted for 8-hours and then transported lost 9.4% bodyweight following the 8-hour journey, while non-fasted and transported animals (NF+T) lost 7.2%. The control animals remaining at grass and non-fasted (NF+G) gained 2%. The animals that were fasted continuously and not transported (F+F) and the non-fasted control animals that were fasted for 8 hours (NF+F) lost 6.1% and 6.2% respectively.

There was no significant change in globulin, glucose, urea, haemoglobin, beta-hydroxy butyrate, fibrinogen concentrations, haematocrit and monocyte percentages, monocyte and red blood cell numbers, platelet numbers among treatments prior to or after transport. The % lymphocytes were reduced in the fasted and non-fasted transported animals and post-transport and there was no significant change in lymphocyte numbers. The % of neutrophils and the number of neutrophils were significantly increased in the fasted and non-fasted transported animals. Baseline protein concentrations were significantly lower in the non-fasted and transported and non-fasted then fasted treatments initially. Following transport, protein concentrations were significantly higher in the fasted and transported treatment compared with the non-fasted animals at grass.

White blood cell (WBC) numbers were not significantly different prior to transport. Following transport, the WBC numbers were significantly higher in the fasted and transported treatment compared with the non-fasted at grass, fasted and then fasted, and the

non-fasted and fasted treatments. Albumin concentrations were significantly higher following transport in the F+T treatment compared with the NF+G, F+F, and NF+F treatments and the NF+T treatment had significantly lower albumin levels than the F+T and NF+F treatments. Haptoglobin concentrations were not significantly different prior to transport. Following transport, haptoglobin concentrations were significantly higher in the F+T compared with the NF+G treatment. Lactate concentrations were significantly higher in the F+T and NF+T compared with the NF+G, F+F, and NF+F treatments following transport.

In conclusion, from the physiological and haematological measurements, an 8 hour journey time, even without access to feed for 8 hours prior to transport did not impact negatively on animal welfare.

## 2. 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 hours (CEC 1991), and there is expert opinion that the feeding interval during transport should be reduced to 12 hours (Scientific Committee on Animal Health and Animal Welfare 2002). 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). These periods of pre-movement/transport fasting can be difficult to control, yet there is ample evidence that extended periods of feed withdrawal can result in bodyweight loss and reductions in meat quality in animals that are transported to slaughter (Bass and Duganzich 1980, Smith and others 1982).

Furthermore, feed withdrawal can impact on animal welfare (Warriss 1992), through hunger and metabolic stress. Increases in plasma concentrations of metabolic markers such as lactate, beta-hydroxybutyrate and urea have been used to measure the metabolic impacts of transport in cattle (Warriss and others 1995). Alterations in immune function are an additional potential impact of transport that are particularly relevant to younger animals (Blecha and others 1984, Phillips and others 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 hours before a journey (Anonymous, 1994), with a view to reducing faecal output during transport, and thereby facilitating a more comfortable journey.

## 3. OBJECTIVES

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

## 4. MATERIALS AND METHODS

The study utilised ninety-two 8-month-old Friesian bull calves that had a mean initial bodyweight of 229.6 ± 32.9 kg and were managed at pasture. The experimental treatments were based around an 8-hour pre-transport fasting period, followed by an 8-hour road journey. The treatments were: 1), Fasted and transported (F+T) (N=20); 2). Non-fasted and transported (NF+T) (N=18); 3). Non-fasted at grass (NF+G) (N=18); 4). Fasted then fasted (F+F) (N=18), and 5), Non-fasted then fasted (NF+F) (N=18). The animals that were subjected to the pre-transport fasting period 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 (F+F; NF+F) were also held in the same area.

The transported bulls were carried on the lower deck of an air sprung articulated transporter (total area = 30.96m$^2$) which was equally divided into 4 fan ventilated pens at a stocking density of 0.82m$^2$/animal. The journey commenced at 21:45, and consisted of 4 hours of travel, followed by a 1-hour rest period, followed by a further 4 hours' travel to the point of origin. The total journey (474km) involved a combination of road surfaces ranging from motorways, secondary roads to small country lanes.

All the animals were blood sampled by jugular venepuncture immediately before the transport period (day 0; Sample 1), and again at the end of the transport period (Sample 2).

# 5. PHYSIOLOGICAL, HAEMATOLOGICAL AND IMMUNOLOGICAL VARIABLES

## 5.1 Body temperature (Rectal 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. The surface body temperatures (°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.

## 5.2 Methodolgy for 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 (CellDyn 3500 Analyser (Abott). 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 (Skinner and Roberts, 1994). Fibrinogen concentrations are measured using a commercial biochemical assay kit (Boehringer Mannheim, Germany). All other physiological measurements were made using Randox assay procedures.

## 6. 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.

# 7. RESULTS AND DISCUSSION

## 7.1 Environmental conditions; temperature, relative humidity during transport

The relative humidity (RH percentage) recorded in the transporter ranged from 74.5 – 94.4%, while the temperature ranged from 11.0 – 15.4°C. The ambient relative humidity and ambient temperature of the transporter ranged from 74.5 – 94.4% and 11.0–15.4°C, respectively. The percentage carbon dioxide, wind velocity and vapour density recorded during transport were 0.06–0.11%; 0.26–0.50m/s and 8.6–13.8td°C respectively. The ambient relative humidity and ambient temperature of the environment ranged from 82.8 – 99.8% and 9.9 – 14.5°C, respectively.

## 7.2 Liveweight

The changes in liveweight are shown in Table 1. There were no significant differences in liveweights among treatments on days 0 (pre-transport), 1 (post-transport), 4 and 10.

Animals fasted and then transported lost 9.4% bodyweight during the 8-hour journey, while NF+T animals lost 7.2%. The animals remaining at grass (NF+G) gained 2%. The F+F and NF+F treatments lost 6.1% and 6.2%, respectively. By Day 10 of the study, the liveweights of all the animals were significantly higher than their pre-transport values.

## 7.3 Body temperature and Surface Temperature

There were no significant differences in rectal body temperature and surface temperatures among treatments on days 0 (pre-transport) (Table 2 and Table 3). Following transport NF+T animals had significantly lower body temperature when compared with pre-transport values and NF+G and NF+F animals had significantly lower rectal body temperature when compared with baseline values. On Day 10 of the study, there were no significant differences in rectal body temperature among treatments, however, readings

were significantly lower for F+T animals, higher for NF+G, F+T and NF+F animals compared with pre-transport baseline readings.

## 7.4 Physiological, haematological and immunological variables

### 7.4.1 Globulin

There was no significant change in globulin concentrations among treatments prior to or after transport (Table 4). Using a paired t-test comparison of sample 2 with sample 1 for each treatment, globulin concentrations were significantly higher in the F+T, NF+T, and NF+F treatments.

### 7.4.2 Glucose

There was no significant change in glucose concentrations among treatments prior to transport (Table 4). Plasma glucose concentrations were significantly higher post-transport in the F+T and NF+T treatments. Using a paired t-test comparison of sample 2 with sample 1 for each treatment, glucose concentrations were significantly higher in the F+T, NF+T, and F+F treatments.

### 7.4.3 Haemoglobin

There was no significant change in Haemoglobin concentrations (Table 4), among treatments prior to or after transport. Using a paired t-test comparison of sample 2 with sample 1 for each treatment, haemoglobin concentrations were significantly higher in the F+T, NF+T, and F+F treatments.

### 7.4.4 Mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC) and mean cell volume (MCV)

There was no significant change in mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC) and mean cell volume (MCV) among treatments prior to or after transport (Table 5).

### 7.4.5 Lymphocyte % and Numbers

The % lymphocytes were reduced in the transported animals (F+T and NF+T) post-transport and there was no significant change in

lymphocyte numbers (Table 6). Using a paired t-test comparison of sample 2 with sample 1 for each treatment, the lymphocyte % was significantly lower in the F+T, NF+T and the F+T treatments and the number of lymphocytes were significantly decreased in the F+T, NF+T, NF+G and NF+F treatments.

### 7.4.6 Neutrophils % and Numbers

The % of neutrophils and the number of neutrophils were significantly increased in the F+T and NF+T animals after transport (Table 7). Using a paired t-test comparison of sample 2 with sample 1 for each treatment, the % neutrophils was significantly increased in the F+T and NF+T treatments and decreased in the F+F treatment.

### 7.4.7 Platelets

There was no significant change in platelet numbers among treatments prior to or after transport (Table 7). Using a paired t-test comparison of sample 2 with sample 1 for each treatment, platelet numbers were significantly higher in the F+T, and lower in the NF+F treatments.

### 7.4.8 Protein

Baseline protein concentrations were significantly lower in the NF+T and NF+F treatments at sample 1 (Table 8). Following transport, protein concentrations were significantly higher in the F+T treatment compared with the NF+G treatment. There was no significant change in protein concentrations in the other treatments. Using a paired t-test comparison of sample 2 with sample 1 for each treatment, protein concentrations were significantly higher in the F+T, NF+T, F+F and NF+F treatments.

### 7.4.9 Haematocrit %

There was no significant change in haematocrit % prior to or after transport (Table 8). Using a paired t-test comparison of sample 2 with sample 1 for each treatment, haematocrit % were significantly higher in the F+T, NF+T, F+F treatments and lower in the NF+G treatment.

### 7.4.10 UREA

Urea concentrations were not changed significantly at any time during the experiment (Table 9). Using a paired t-test comparison of sample 2 with sample 1 for each treatment, urea concentrations were significantly lower in the NF+T treatment.

### 7.4.11 White blood cell numbers

White blood cell (WBC) numbers were not significantly different prior to transport (Table 9). Following transport, the WBC numbers were significantly higher in the F+T treatment compared with the NF+G, F+F, and NF+F treatments. Using a paired t-test comparison of sample 2 with sample 1 for each treatment, WBC numbers were significantly higher in the F+T, NF+T, NF+G treatments and lower in the F+F and NF+F treatments.

### 7.4.12 Albumin

Albumin concentrations were significantly lower in the NF+F at sample 1 (Table 10). Following transport, albumin concentrations were significantly higher in the F+T treatment compared with the NF+G, F+F, and NF+F treatments and the NF+T treatment had significantly lower albumin levels than the F+T and NF+F treatments. Using a paired t-test comparison of sample 2 with sample 1 for each treatment, albumin concentrations were significantly higher in the F+T, NF+T and NF+F treatments.

### 7.4.13 Betahydroxybutyrate (BHB)

BHB concentrations were not significantly changed at any time during the experiment (Table 10). Using a paired t-test comparison of sample 2 with sample 1 for each treatment, BHB concentrations were significantly lower in the NF+T and NF+F treatment and significantly higher in the F+T and F+F treatments respectively.

### 7.4.14 Fibrinogen

Fibrinogen concentrations were not significantly different prior to or after transport (Table 11). Using a paired t-test comparison of sample 2 with sample 1 for each treatment, fibrinogen concentrations were significantly lower in the F+T and NF+G treatments.

### 7.4.15 Haptoglobin

Haptoglobin concentrations were not significantly different prior to transport (Table 11). Following transport, haptoglobin concentrations were significantly higher in the F+T compared with the NF+G treatment. Using a paired t-test comparison of sample 2 with sample 1 for each treatment, haptoglobin concentrations were significantly lower in the NF+G treatment.

### 7.4.16 Lactate

Lactate concentrations were not significantly different prior to transport (Table 11). Following transport, Lactate concentrations were significantly higher in the F+T and NF+T compared with the NF+G, F+F, and NF+F treatments. Using a paired t-test comparison of sample 2 with sample 1 for each treatment, albumin concentrations were significantly higher in the F+T, treatment and lower in the NF+G and NF+F treatments.

### 7.4.17 Monocyte % and monocyte numbers

The percentage and number of monocytes were not significantly changed at any time during the experiment (Table 12).

### 7.4.18 Red blood cell numbers

Red blood cell (RBC) numbers were not significantly different prior to or following transport (Table 12). Using a paired t-test comparison of sample 2 with sample 1 for each treatment, RBC numbers were significantly higher in the F+T, and NF+F treatments, respectively.

## 8. Conclusion

The results suggest that from the physiological and haematological measurements, an 8 hour journey time, even without access to feed for 8 hours prior to transport did not impact negatively on animal welfare. βeta-hydroxybutyrate and urea were not changed at any time throughout the experiment. The increase in glucose concentrations in F+T and NF+T treatments could have been due to catecholamine-stimulated glycogenolysis. An increase in neutrophils and decrease in lymphocyte numbers, increases in white blood cell counts 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 and others 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.

The influence of the type of animal transported upon transport-induced stress response was investigated by Tennessen and others (1984). In their studies, heart rates, serum cortisol concentrations and body temperatures were measured over a series of seven journeys in bulls (513 kg bodyweight) and steers (473 kg bodyweight). The cortisol response to transport and increase in body temperature was found to be greater for steers than bulls over 2-h journeys, and Tennessen and others (1984) reported similar and minimal overall responses for both sets of animals. In the present study, bulls (230kg) undergoing an 8-h transportation at stocking densities of 0.82 $m^2$ /animal showed physiological, haematological and immunological responses that were within normal referenced ranges. The effects of the duration of journey on the response of cattle of different ages to transportation has been the subject of several studies.

Mormede and others (1982) 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 wk after transport. In the present study, albumin concentrations increased in the Fasted and transported bulls following transport with no change in haematocrit percentages. Kent and Ewbank (1986a) transported calves (1- to 3-wk-old) by road for either 6 or 18 h. In the present study, the % of neutrophils and the number of neutrophils were significantly increased in the F+T and NF+T animals after transport. In comparison with control calves that were not transported, but starved for 18 h, transported calves had increased neutrophil and decreased lymphocyte numbers. Mormede and others (1982), Kent and Ewbank (1986a) recorded no evidence of dehydration in calves transported for up to 18 h, as measured by plasma protein concentration and packed cell volume. Calves transported for 6 h became hypoglycaemic when they were not fed for a further 12 h, but there were few biochemical differences and no difference in proportion of time spent lying between calves transported for either 6 or 18 h (Kent and Ewbank, 1986a). In a subsequent study (Kent and Ewbank, 1986b), 3-mo-old calves were similarly transported for either 6 or 18 h, or starved for 18 h without being transported. Plasma glucose concentrations were elevated in transported calves compared with starved controls, as were plasma cortisol, non-esterified fatty acids and total white blood cell numbers. Plasma non-esterified fatty acid concentrations were elevated for longer post transport in calves transported for 18 h compared with 6 h, but there were no other differences in biochemical measurements related to journey duration. Warriss and others (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. Animals that were transported for 5, 10 and 15 h lost 4.6, 6.5 and 7.0% of their BW, respectively; and recovery to pre-transport BW generally took 5 d. Plasma cortisol concentrations were increased by loading, but not by journey duration.

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. It was concluded by Warriss and others (1995) that under the conditions of their study, a 15-h journey by road for 12- to 18-mo-old cattle did not impinge negatively on the welfare of the animals.

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 d (Schaefer and others 1988); and with the suppression of reproductive function (Nanda and others 1989). The physiological responses of cattle to transport have been documented by Leach (1981) while Shaw and Tume (1992) have reported the effects of preslaughter handling on the plasma levels of blood constituents.

Transport conditions have the potential to alter the physiological responses of young bulls to the psychological or physical stress of transport. In conclusion, the results of this study show that from the physiological and haematological measurements, an 8 hour journey time, even without access to feed for 8 hours prior to transport did not impact negatively on animal welfare.

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**Table 1: Changes in liveweight in fasted and non-fasted control and transported animals. Values are expressed as mean (kg) ± SD with P values.**

| Treatment | Stat | Day 0 | Day 1 | Day 4 Day 10 | |
|---|---|---|---|---|---|
| Fasted + transported | Mean | 227.5 | 206.1 | 229.2 | 239.4 |
| | SD | 23.74 | 22.54 | 23.02 | 24.64 |
| | Compare to Day 1 | | P = 0.0001 | P = 0.1199 | P = 0.0001 |
| Non-fasted and | Mean | 231.2 | 214.6 | 230.7 | 242.9 |
| transported | SD | 35.52 | 36.33 | 32.92 | 36.78 |
| | Compare to Day 1 | | P = 0.0001 | P = 0.7255 | P = 0.0001 |
| Non-fasted at grass | Mean | 226.1 | 230.1 | 226.3 | 239.6 |
| | SD | 40.56 | 41.73 | 40.09 | 42.3 |
| | Compare to Day 1 | | P = 0.0308 | P = 0.9302 | P = 0.0001 |
| Fasted then fasted | Mean | 230.7 | 216.7 | 233.6 | 242.8 |
| | SD | 29.3 | 28.33 | 31.24 | 30.92 |
| | Compare to Day 1 | | P = 0.0001 | P = 0.0261 | P = 0.0001 |
| Non-fasted then fasted | Mean | 233.2 | 218.8 | 228.8 | 240.9 |
| | SD | 35.78 | 29.67 | 30.04 | 32.26 |
| | Compare to Day 1 | | P = 0.0001 | P = 0.1444 | P = 0.0043 |
| | Tukey Sig. | NS | NS | NS | NS |

| Repeated Sig. P = | |
|---|---|
| Treat | 0.9846 |
| Weight | 0.0001 |
| Trt * Wgt | 0.0001 |

**Table 2: Changes in rectal body temperature in fasted and non-fasted control and transported animals. Values are expressed as mean (kg) ± SD with P values.**

| Treatment | Statistic | Day 0 | Day 1 | Day 10 |
|---|---|---|---|---|
| Fasted + transported | Mean | 38.6 | 38.4 | 37.1 |
| | SD | 0.458 | 0.285 | 0.342 |
| | Compare to Day 1 | | P = 0.1168 | P = 0.0006 |
| Non-fasted and | Mean | 38.8 | 38.5 | 39.0 |
| transported | SD | 0.371 | 0.347 | 0.214 |
| | Compare to Day 1 | | P = 0.0103 | P = 0.0762 |
| Non-fasted at grass | Mean | 38.8 | 38.5 | 39.0 |
| | SD | 0.359 | 0.264 | 0.289 |
| | Compare to Day 1 | | P = 0.0054 | P = 0.0431 |
| Fasted then fasted | Mean | 38.5 | 38.5 | 39.1 |
| | SD | 0.514 | 0.246 | 0.396 |
| | Compare to Day 1 | | P = 0.7745 | P = 0.0002 |
| Non-fasted then fasted | Mean | 38.9 | 38.6 | 39.1 |
| | SD | 0.249 | 0.486 | 0.437 |
| | Compare to Day 1 | | P = 0.027 | P = 0.0403 |
| | Tukey Sig. | NS | NS | NS |

NS non significant

**Repeated Sig. P =**

| | |
|---|---|
| **Treat** | **0.0987** |
| **Body** | **0.0001** |
| **Trt * Body** | **0.1034** |

**Table 3: Changes in surface body temperature in fasted and non-fasted control and transported animals (day 1). Values are expressed as mean (kg) ± SD with P values.**

| Treatment | Stat | Shoulder | Rump | Belly |
|---|---|---|---|---|
| Fasted + transported | Mean | 24.3 | 23.2 | 24.0 |
| | SD | 3.076 | 1.95 | 3.156 |
| Non-fasted + transported | Mean | 24.7 | 22.2 | 23.8 |
| | SD | 5.199 | 2.677 | 3.05 |
| Non-fasted at grass | Mean | 24.9 | 25.3 | 25.7 |
| | SD | 2.292 | 2.234 | 2.107 |
| Fasted then fasted | Mean | 25.5 | 24.7 | 24.1 |
| | SD | 2.387 | 4.318 | 3.999 |
| Non-fasted then fasted | Mean | 25.5 | 24.8 | 24.0 |
| | SD | 2.101 | 3.047 | 1.882 |
| | Tukey Sig. | NS | NS | NS |

| Repeated Sig. P = | |
|---|---|
| Treat | 0.0902 |
| Surface | 0.0501 |
| Trt * Surface | 0.2618 |

**Table 4:** **Effect of fasting and non-fasting animals for 8 hours prior to an 8-hour transport journey on physiological and haematological variables taken before (Sample 1) and immediately after (Sample 2) the journey. Values are expressed as Mean ± SD with P values. (Normal Data Analysis of Variance with Tukey Multiple Comparison Test).**

| Variable | Treat | N | Sample 1 Mean | SD | Sample 2 Mean | SD | Paired t-test; P= |
|---|---|---|---|---|---|---|---|
| Globulin | Fasted +transported | 20 | 41.53 | 3.807 | 43.53 | 4.238 | 0.0005 |
| g/l | Non-Fasted + transported | 18 | 39.93 | 3.823 | 42.81 | 4.422 | 0.0001 |
| | Non-fasted at grass | 18 | 41.48 | 4.674 | 41.59 | 4.863 | 0.7966 |
| | Fasted then fasted | 18 | 42.93 | 3.825 | 43.72 | 3.727 | 0.0850 |
| | Non-fasted then fasted | 18 | 40.64 | 5.239 | 42.47 | 5.080 | 0.0001 |
| | | | Significance P = 0.3048 | | P = 0.6167 | | |
| | Tukey | | NS | | NS | | |
| Glucose | Fasted + transported | 20 | 4.35 | 0.287 | 4.96 | 0.289 | 0.0001 |
| mmol/l | Non-Fasted + transported | 18 | 4.17 | 0.392 | 4.98 | 0.517 | 0.0001 |
| | Non-fasted at grass | 18 | 4.14 | 0.273 | 4.14* | 0.189 | 1.0000 |
| | Fasted then fasted | 18 | 4.33 | 0.323 | 4.01* | 0.305 | 0.0001 |
| | Non-fasted then fasted | 18 | 4.11 | 0.291 | 4.14* | 0.418 | 0.6924 |
| | | | Significance P = 0.0751 * | | P = 0.0001 | | |
| | Tukey | | NS | | Vs F+T; NF+T | | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Haemoglobin | Fasted + transported | 20 | 11.24 | 0.771 | 11.71 | 0.679 | 0.0001 |
| g% | Non-fasted + transported | 18 | 11.21 | 0.653 | 11.45 | 0.807 | 0.0283 |
| | Non-fasted at grass | 18 | 11.53 | 0.869 | 11.19 | 0.694 | 0.0256 |
| | Fasted then fasted | 18 | 11.14 | 0.575 | 11.46 | 0.752 | 0.0179 |
| | Non-fasted then fasted | 18 | 11.12 | 0.933 | 11.21 | 0.962 | 0.5011 |
| | | | Significance P = 0.5096 | | P = 0.2446 | | |
| | Tukey | | NS | NS | | | |

NS Non significant

**Table 5:** **Effect of fasting and non-fasting animals for 8 hours prior to an 8-hour transport journey on physiological and haematological variables taken before (Sample 1) and immediately after (Sample 2) the journey. Values are expressed as Mean ± SD with P values. (Normal Data Analysis of Variance with Tukey Multiple Comparison Test).**

| Variable | Treat | N | Sample 1 Mean | SD | Sample 2 Mean | SD | Paired t-test; P= |
|---|---|---|---|---|---|---|---|
| Mean cell | Fasted + transported | 20 | 11.42 | 0.697 | 11.39 | 0.678 | 0.4449 |
| haemoglobin | Non-fasted + transported | 18 | 11.76 | 0.617 | 11.67 | 0.630 | 0.0311 |
| (MCH) pg | Non-fasted at grass | 18 | 11.59 | 0.751 | 11.47 | 0.693 | 0.0644 |
| | Fasted then fasted | 18 | 11.63 | 0.732 | 11.52 | 0.742 | 0.0031 |
| | Non-fasted then fasted | 18 | 11.54 | 0.810 | 11.57 | 0.836 | 0.7654 |
| | | Significance | 0.6917 | | 0.8048 | | |
| | Tukey | NS | NS | | | | |
| MCHC | Fasted + transported | 20 | 33.71 | 0.621 | 33.86 | 0.477 | 0.2392 |
| g/dl | Non-fasted + transported | 18 | 33.96 | 0.614 | 33.70 | 0.686 | 0.0332 |
| | Non-fasted at grass | 18 | 33.61 | 0.562 | 33.84 | 0.468 | 0.0483 |
| | Fasted then fasted | 18 | 33.47 | 0.777 | 33.39 | 0.801 | 0.4251 |
| | Non-fasted then fasted | 18 | 33.52 | 0.599 | 33.50 | 0.493 | 0.8550 |
| | | Significance | 0.1622 | | 0.0754 | | |
| | Tukey | NS | NS | | | | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| MCV | Fasted + transported | 20 | 33.92 | 1.927 | 33.62 | 1.831 | 0.0005 |
| fl | Non-fasted + transported | 18 | 34.64 | 1.915 | 34.64 | 1.974 | 1.0000 |
| | Non-fasted at grass | 18 | 34.46 | 2.232 | 33.93 | 2.133 | 0.0248 |
| | Fasted then fasted | 18 | 34.73 | 2.356 | 34.55 | 2.389 | 0.0246 |
| | Non-fasted then fasted | 18 | 34.43 | 2.202 | 34.52 | 2.371 | 0.6671 |
| | | Significance | 0.7907 | | 0.5048 | | |
| | Tukey | NS | NS | | | | |

NS Non significant

**Table 6: Effect of fasting and non-fasting animals for 8 hours prior to an 8-hour transport journey on physiological and haematological variables taken before (Sample 1) and immediately after (Sample 2) the journey. Values are expressed as Mean ± SD with P values. (Normal Data Analysis of Variance with Tukey Multiple Comparison Test).**

| Variable | Treat | N | Sample 1 Mean | SD | Sample 2 Mean | SD | Paired t-test; P= |
|---|---|---|---|---|---|---|---|
| Lymphocyte % | Fasted + transported | 20 | 47.2 | 9.52 | 34.5 | 7.88 | 0.0001 |
| | Non-fasted + transported | 18 | 48.8 | 10.66 | 36.4 | 11.44 | 0.0014 |
| | Non-fasted at grass | 18 | 48.6 | 6.35 | 48.9* | 6.34 | 0.8234 |
| | Fasted then fasted | 18 | 45.1 | 9.57 | 50.4* | 7.85 | 0.0418 |
| | Non-fasted then fasted | 18 | 49.9 | 10.19 | 51.3* | 9.61 | 0.5369 |
| | | Significance | 0.5807 | | *P = 0.0001 | | |
| | Tukey | | NS | | Vs F+T ; NF+T | | |
| Lymphocyte No | Fasted + transported | 20 | 6.319 | 1.1248 | 5.279 | 1.171 | 0.0006 |
| | Non-fasted + transported | 18 | 6.407 | 1.3348 | 5.348 | 1.6198 | 0.0101 |
| | Non-fasted at grass | 18 | 7.110 | 1.3492 | 6.249 | 1.2935 | 0.0132 |
| | Fasted then fasted | 18 | 5.576 | 1.2288 | 5.367 | 1.1081 | 0.4903 |
| | Non-fasted then fasted | 18 | 6.825 | 1.5002 | 6.004 | 1.4334 | 0.0052 |
| | | Significance | 0.0096 | | 0.0954 | | |
| | Tukey | | 3,5 > 4 | | NS | | |

NS Non significant

**Table 8:** **Effect of fasting and non-fasting animals for 8 hours prior to an 8-hour transport journey on physiological and haematological variables taken before (Sample 1) and immediately after (Sample 2) the journey. Values are expressed as Mean ± SD with P values. (Normal Data Analysis of Variance with Tukey Multiple Comparison Test).**

| Variable | Treat | N | Sample 1 Mean | SD | Sample 2 Mean | SD | Paired t-test; P= |
|---|---|---|---|---|---|---|---|
| Protein g/l | Fasted + transported | 20 | 77.66 | 3.256 | 81.33 | 4.122 | 0.0001 |
| | Non-fasted + transported | 18 | 74.78* | 3.243 | 79.49 | 4.478 | 0.0001 |
| | Non-fasted at grass | 18 | 76.37 | 4.735 | 76.81* | 4.974 | 0.3197 |
| | Fasted then fasted | 18 | 78.53 | 4.010 | 79.68 | 4.504 | 0.0543 |
| | Non-fasted then fasted | 18 | 74.70* | 4.190 | 77.49 | 4.359 | 0.0001 |
| | | Significance | * P = 0.0112 | | * P = 0.0199 | | |
| | Tukey | | Vs F+F Sample 1 | | Vs F+T Sample 2 | | |
| Haematocrit % | Fasted + transported | 20 | 33.39 | 2.225 | 34.58 | 1.934 | 0.0007 |
| | Non-fasted + transported | 18 | 33.00 | 1.768 | 34.02 | 2.360 | 0.0014 |
| | Non-fasted at grass | 18 | 34.31 | 2.820 | 33.08 | 2.094 | 0.0151 |
| | Fasted then fasted | 18 | 33.33 | 1.920 | 34.33 | 2.231 | 0.0161 |
| | Non-fasted then fasted | 18 | 33.23 | 2.893 | 33.44 | 2.862 | 0.6258 |
| | | Significance | P = 0.5204 | | P = 0.2605 | | |
| | Tukey | | NS | | NS | | |

NS Non significant

**Table 7:** **Effect of fasting and non-fasting animals for 8 hours prior to an 8-hour transport journey on physiological and haematological variables taken before (Sample 1) and immediately after (Sample 2) the journey. Values are expressed as Mean ± SD with P values. (Normal Data Analysis of Variance with Tukey Multiple Comparison Test).**

| | | | Sample 1 | | Sample 2 | | |
|---|---|---|---|---|---|---|---|
| Variable | Treat | N | Mean | SD | Mean | SD | Paired t-test; P= |
| Neutrophil % | Fasted + transported | 20 | 50.6 | 10.63 | 63.8 | 7.75 | 0.0001 |
| | Non-fasted + transported | 18 | 48.6 | 10.74 | 61.1 | 11.95 | 0.0027 |
| | Non-fasted at grass | 18 | 48.6 | 6.81 | 48.3* | 6.70 | 0.8928 |
| | Fasted then fasted | 18 | 52.3 | 9.64 | 46.6* | 7.97 | 0.0348 |
| | Non-fasted then fasted | 18 | 46.7 | 10.98 | 46.0* | 10.40 | 0.7656 |
| | | Significance | P = 0.4963 | | *P = 0.0001 | | |
| | Tukey | | NS | | Vs F+T; NF+T | | |
| Neutrophil No | Fasted + transported | 20 | 7.140 | 2.4959 | 9.986 | 2.2967 | 0.0001 |
| | Non-fasted + transported | 18 | 6.659 | 2.5746 | 9.334 | 2.9751 | 0.0042 |
| | Non-fasted at grass | 18 | 7.281 | 2.2028 | 6.383* | 2.1313 | 0.0224 |
| | Fasted then fasted | 18 | 6.892 | 2.1194 | 4.977* | 1.231 | 0.0007 |
| | Non-fasted then fasted | 18 | 6.603 | 2.3488 | 5.449* | 1.7697 | 0.0097 |
| | | Significance | P = 0.8865 | | *P = 0.0001 | | |
| | Tukey | | NS | | Vs F+T; NF+T | | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Platelets | Fasted + transported | 20 | 868.2 | 259.50 | 920.1 | 247.16 | 0.0154 |
| $10^9/l$ | Non-fasted + transported | 18 | 914.1 | 289.34 | 903.6 | 306.32 | 0.6861 |
| | Non-fasted at grass | 18 | 970.4 | 290.38 | 957.2 | 281.61 | 0.7116 |
| | Fasted then fasted | 18 | 868.6 | 289.69 | 835.7 | 285.09 | 0.2183 |
| | Non-fasted then fasted | 18 | 861.6 | 220.08 | 798.4 | 220.49 | 0.0110 |
| | | Significance | P = 0.7133 | | P = 0.3891 | | |
| | Tukey | | NS | | NS | | |

NS Non significant

**Table 9: Effect of fasting and non-fasting animals for 8 hours prior to an 8-hour transport journey on physiological and haematological variables taken before (Sample 1) and immediately after (Sample 2) the journey. Values are expressed as Mean ± SD with P values. (Normal Data Analysis of Variance with Tukey Multiple Comparison Test).**

| Variable | Treat | N | Sample 1 Mean | SD | Sample 2 Mean | SD | Paired t-test; P= |
|---|---|---|---|---|---|---|---|
| UREA mmol/l | Fasted + transported | 20 | 5.07 | 1.045 | 5.06 | 0.672 | 0.9345 |
| | Non-fasted + transported | 18 | 5.45 | 1.049 | 4.71 | 0.749 | 0.0001 |
| | Non-fasted at grass | 18 | 5.49 | 1.041 | 5.43 | 1.147 | 0.7234 |
| | Fasted then fasted | 18 | 5.27 | 0.634 | 5.37 | 0.581 | 0.3054 |
| | Non-fasted then fasted | 18 | 5.33 | 0.801 | 5.39 | 0.761 | 0.6262 |
| | | Significance | P = 0.6517 | | P = 0.0363 | | |
| | Tukey | | NS | | too small for multiple comparison | | |
| White blood cell | Fasted+transported | 20 | 13.75 | 2.810 | 15.54 | 2.551 | 0.0001 |
| No $X10^3$ µl | Non-fasted + transported | 18 | 13.42 | 2.794 | 15.06** | 2.456 | 0.0091 |
| | Non-fasted at grass | 18 | 14.81 | 3.055 | 12.97*^ | 3.204 | 0.0029 |
| | Fasted then fasted | 18 | 12.98 | 2.024 | 10.66* | 1.574 | 0.0001 |
| | Non-fasted then fasted | 18 | 13.91 | 2.650 | 11.76* | 2.102 | 0.0005 |
| | | Significance | P = 0.3432 | | P = 0.0001 | | |
| | Tukey | | NS | | *Vs F+T; ** Vs F+F and NF+F; ^ Vs F+F | | |

NS Non significant

**Table 10: Effect of fasting and non-fasting animals for 8 hours prior to an 8-hour transport journey on physiological and haematological variables taken before (Sample 1) and immediately after (Sample 2) the journey. Values are expressed as Median with minimum and maximum values and P values (Non Parametric Kruskal-Wallis Test with Tukey Multiple Comparison Test).**

| Variable | Treat | N | Sample 1 Median | Min–Max | Sample 2 Median | Min–Max | Wilcoxon Signed Rank P= |
|---|---|---|---|---|---|---|---|
| Albumin | Fasted + transported | 20 | 36.35 | 32.3–38.3 | 37.60 | 35.7–40.4 | 0.0001 |
| g/l | Non-fasted + transported | 18 | 34.90 | 31.6–37.7 | 36.75** | 35.2–38.7 | 0.0001 |
| | Non-fasted at grass | 18 | 35.20 | 29.4.–37.6 | 35.40* | 29.6–38.3 | 0.2501 |
| | Fasted then fasted | 18 | 35.70 | 33.9–37.0 | 35.75* | 32.7–37.9 | 0.1262 |
| | Non-fasted then fasted | 18 | 34.35* | 27.7–38.0 | 35.45* | 30.2–37.8 | 0.0053 |
| | | Significance | *P = 0.0089 | | P = 0.0001 | | |
| | Tukey | | Vs F+T * | | Vs F+T; **Vs NF+G and NF+F | | |
| BHB g/l | Fasted + transported | 20 | 0.170 | 0..09–0.36 | 0.260* | 0.13–0.47 | 0.0153 |
| | Non-fasted + transported | 18 | 0.400 | 0.20–0.62 | 0.300 | 0.15–0.52 | 0.0029 |
| | Non-fasted at grass | 18 | 0.385* | 0.29–0.48 | 0.360 | 0.26–0.53 | 0.3970 |
| | Fasted then fasted | 18 | 0.185* | 0.07–0.35 | 0.275* | 0.20–0.48 | 0.0005 |
| | Non-fasted then fasted | 18 | 0.390* | 0.23–0.55 | 0.265* | 0.11–0.35 | 0.0001 |
| | | Significance | * P = 0.0001 | | * P = 0.0001 | | |
| | Tukey | | Vs F+T; NF+T | | Vs NF+G | | |

NS Non significant

**Table 11: Effect of fasting and non-fasting animals for 8 hours prior to an 8-hour transport journey on physiological and haematological variables taken before (Sample 1) and immediately after (Sample 2) the journey. Values are expressed as Median with minimum and maximum values and P values (Non Parametric Kruskal-Wallis Test with Tukey Multiple Comparison Test).**

| Variable | Treat | N | Sample 1 Median | Min–Max | Sample 2 Median | Min–Max | Wilcoxon Signed Rank P= |
|---|---|---|---|---|---|---|---|
| Fibrinogen | Fasted + transported | 20 | 682.0 | 514–897 | 634.5 | 548–785 | 0.0336 |
| mg/dl | Non-fasted + transported | 18 | 630.5 | 488–988 | 640.0 | 526–1094 | 0.1160 |
| | Non-fasted at grass | 18 | 661.0 | 571–1065 | 624.0 | 486–1031 | 0.0155 |
| | Fasted then fasted | 18 | 668.0 | 443–793 | 635.0 | 461–839 | 0.1964 |
| | Non-fasted then fasted | 18 | 664.0 | 529–990 | 634.5 | 512–783 | 0.7467 |
| | | Significance | P = 0.4482 | | P = 0.8597 | | |
| | Tukey | | NS | | NS | | |
| Haptoglobin | Fasted + transported | 20 | 0.200 | 0.12–0.78 | 0.235 | 0.11–0.76 | 0.1545 |
| g Hb/l | Non-fasted + transported | 18 | 0.160 | 0.12–0.33 | 0.175 | 0.10–0.44 | 0.5234 |
| | Non-fasted at grass | 18 | 0.180 | 0.12–2.27 | 0.140* | 0.10–1.95 | 0.0036 |
| | Fasted then fasted | 18 | 0.175 | 0.12–0.56 | 0.175 | 0.12–0.52 | 0.3250 |
| | Non-fasted then fasted | 18 | 0.175 | 0.11–0.87 | 0.170 | 0.10–0.62 | 0.2444 |
| | | Significance | P = 0.3792 | | * P = 0.0054 | | |
| | Tukey | | NS | | Vs F+T | | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Lactate | Fasted + transported | 20 | 0.535 | 0.35–1.45 | 0.955 | 0.51–2.30 | 0.0008 |
| mmol/l | Non-fasted + transported | 18 | 0.645 | 0.35–2.03 | 0.735 | 0.46–2.68 | 0.0552 |
| | Non-fasted at grass | 18 | 0.630 | 0.46–3.19 | 0.485* | 0.28–0.89 | 0.0134 |
| | Fasted then fasted | 18 | 0.515 | 0.36–1.08 | 0.540* | 0.31–1.86 | 0.7097 |
| | Non-fasted then fasted | 18 | 0.575 | 0.34–2.30 | 0.445* | 0.34–1.14 | 0.0229 |
| | | Significance | P = 0.2868 | | * P = 0.0001 | | |
| | Tukey | | NS | | Vs F+T; NF+T | | |

NS Non significant

**Table 12: Effect of fasting and non-fasting animals for 8 hours prior to an 8-hour transport journey on physiological and haematological variables taken before (Sample 1) and immediately after (Sample 2) the journey. Values are expressed as Median with minimum and maximum values and P values (Non Parametric Kruskal-Wallis Test with Tukey Multiple Comparison Test).**

| Variable | Treat | N | Sample 1 Median | Min–Max | Sample 2 Median | Min–Max | Wilcoxon Signed Rank P= |
|---|---|---|---|---|---|---|---|
| Monocyte | Fasted + transported | 20 | 1.5 | 0–4 | 1.0 | 0–5 | 0.7891 |
| | Non-fasted + transported | 18 | 1.0 | 0–4 | 2.0 | 0–5 | 0.1658 |
| | Non-fasted at grass | 18 | 2.0 | 0–5 | 2.0 | 0–4 | 0.6809 |
| | Fasted then fasted | 18 | 2.0 | 1–5 | 2.5 | 1–4 | 0.8608 |
| | Non-fasted then fasted | 18 | 1.5 | 0–7 | 2.0 | 0–5 | 0.8403 |
| | | Significance | P = 0.2866 | | P = 0.2666 | | |
| | Tukey | | NS | | NS | | |
| Monocyte | No Fasted + transported | 20 | 0.185 | 0–0.65 | 0.185 | 0–0.95 | 0.9740 |
| X $10^9$/l | Non-fasted + transported | 18 | 0.170 | 0–0.48 | 0.325 | 0–0.70 | 0.0663 |
| | Non-fasted at grass | 18 | 0.300 | 0–0.76 | 0.225 | 0–0.40 | 0.3286 |
| | Fasted then fasted | 18 | 0.280 | 0.1–0.67 | 0.265 | 0.09–0.44 | 0.6860 |
| | Non-fasted then fasted | 18 | 0.195 | 0–1.25 | 0.255 | 0–0.53 | 0.5520 |
| | | Significance | P = 0.3041 | | P = 0.6611 | | |
| | Tukey | | NS | | NS | | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Red blood cell | Fasted + transported | 20 | 9.910 | 8.59–10.90 | 10.400 | 9.22–11.30 | 0.0001 |
| No I X $10^6$ ml | Non-fasted + transported | 18 | 9.450 | 8.21–11.40 | 9.605 | 8.13–11.30 | 0.0045 |
| | Non-fasted at grass | 18 | 10.100 | 7.68–11.00 | 9.640 | 8.08–11.10 | 0.1769 |
| | Fasted then fasted | 18 | 9.690 | 8.54–11.00 | 9.925 | 8.62–11.20 | 0.0088 |
| | Non-fasted then fasted | 18 | 9.980 | 7.41–10.80 | 9.930 | 6.90–11.40 | 0.4592 |
| | | Significance | P = 0.2481 | | P = 0.2321 | | |
| | Tukey | | NS | | NS | | |

NS Non significant