ANIMAL HEALTH AND WELFARE

Performance, hoof condition and dirt scores of finishing beef steers on different floor types
There is a shortage of published research on the effects of placing rubber mats on slats in facilities for winter finishing beef cattle. The objective of this study was to investigate the effect of placing mats on concrete slatted floors on performance, hoof condition and dirt scores of finishing beef steers, and to compare responses with animals on out-wintering pads.

Continental crossbred beef steers (n = 180; mean initial liveweight = 513 (s.d. 39.0) kg were blocked by breed and liveweight and were randomly assigned to one of five treatments in November 2006: 1) Concrete slats alone, 2) Mat 1, 3) Mat 2, 4) Mat 3 and 5) Out-wintering pads (OWP's) (constructed in 2006). The mats were supplied and fitted in 2006 by the suppliers. Animals assigned to treatments 1-4 were accommodated in a roofed building with concrete slatted floors. The slats (5 gang/pen) in the individual pens were supplied by Banagher Concrete Ltd., Offaly. The mats were placed over the concrete slats. There were four pens per treatment, with nine steers per pen at a mean space allowance of 2.73m^2/head, whereas animals accommodated outdoors on the OWP's had a space allowance of 12m^2/head.

Blood samples were collected by jugular venipuncture, and dirt scores and live weights were taken on days 0, 21, 42, 62, 84, 105, 126 and 152. A pathological examination of all hooves was made prior to and at the end of the study. All animals were fed a total mixed ration (TMR) comprising of grass silage and a barley based concentrate on a 50:50 dry matter (DM) basis. Animals were slaughtered after 153 - 154 days. Post-slaughter carcass weight, kill-out proportion and kidney and channel fat weight was determined. The pen of cattle was the experimental unit for intake related measurements. Performance data were subjected to ANOVA using a model that accounted for treatment and replicate blocks. Physiological and haematological measurements were tested in a one-way ANOVA (repeated measures) using PROC MIXED, using a means statement with a Tukey option to detect treatment differences. Dirt scores and hoof scores were not normally distributed and were analysed by PROC GLM, using ranked data, in a Kruskall-Wallis test and a Wilcoxon signed rank test to detect treatment differences.

Animals on the OWP's had a higher ($P<0.05$) dry matter (DM) feed intake compared with those on the slats and three mat types (Table 60). Animals on the OWP treatment had a greater liveweight gain ($P<0.05$) than Mat 2 and Mat 3, with slats and Mat 1 being intermediate but not different ($P>0.05$). There was no overall effect ($P>0.05$) of treatment on liveweight or no treatment × time interaction ($P>0.05$). The carcass weight was greater ($P<0.05$) for the OWP and Mat 1 treatments than the slats treatment, with Mat 2 and 3 being intermediate but not different ($P>0.05$). Kill out proportion was higher ($P<0.05$) in all treatments compared with slats. Feed conversion efficiency (FCE) was lower ($P<0.05$) for Mat 1 treatment compared with slats and was not different ($P>0.05$) from Mat 2, Mat 3 and OWP's. No incidence of laminitis was observed among treatments. The number of hoof lesions was higher on all mat types ($P<0.05$) compared with slats and OWP treatments. Dirt scores did not differ ($P>0.05$) between treatments.

Table 60: Intake, performance, slaughter traits, hoof condition and dirt scores of finishing beef steers on slats, slats plus mats and out-wintering pads (Mean ± s.e.m)

Slats	Mat 1	Mat 2	Mat 3	OWP's
Total DM intake (kg /day)				
9.2[a] ± 0.05	9.5 [a] ±0.29	9.4 [a] ±0.06	9.2 [a] ± 0.07	10.1 [b]± 0.07
Live weight gain (g/day)				
1016 [ab] ± 47	1072 [ab] ± 53	964 [a] ± 51	969 [a] ± 42	1112 [b] ± 59
Slaughter weight (kg)				
668 ± 8.7	676 ± 10.2	661 ± 9.9	661 ± 8.9	684 ± 9.7
Carcass weight (kg)				
357 [a] ± 4.2	374 [b]± 5.7	364 [ab] ± 4.8	366 [ab] ± 4.7	378 [b]±5.2
Kill-out proportion (g/kg)				
537 [a] ±5	554 [b]±5	552 [b]±6	553 [b]±5	553 [b]±6
FCE (DMI/kg carcass gain)				
15.4 [b]± 0.4	13.5 [a] ± 0.6	14.7 [ab]± 0.4	14.3 [ab]± 0.7	14.0 [ab]± 0.4
Hoof scores[1] (lesions)				
22 [a] ± 1.8	38 [b] ± 2.4	37 [b]± 2.5	33 [b]± 1.4	20 [a]± 2.3
Dirt scores[2]				
39.4 ± 0.69	37.6 ± 0.76	39.3 ± 0.74	39.3 ± 0.71	37.4 ± 0.75

[ab]Within row, means with the same superscripts are not significantly different ($P \geq 0.05$). [1] The total number of lesions/animal at the end of the study. [2]Sum of 16 body parts each on a cleanliness scale of 1 (clean) to 5 (dirty).

There was no difference in animal performance and dirt scores among the mat treatments. Although carcass weight was greater in animals housed on OWP's and Mat 1 than those on slats it was not significantly different from Mat 2 or 3. There were a greater number of lesions on the hooves of animals housed on mats compared with slats and OWP treatments.

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Effect of weaning strategy on leukocyte populations and immunological variables in beef suckler calves

Stress associated with weaning has been documented. Activation of the stress response in the bovine contributes to disease susceptibility by altering the cellular immune response. The objective of the study was to determine the effect of weaning strategy on leukocyte populations (neutrophil and lymphocyte sub-types) and acute phase proteins (APPs) (fibrinogen and haptoglobin) in beef suckler calves.

Following abrupt weaning, thirty-six spring-born, beef suckler calves (276, s.d. 37.0 kg) were either i) housed (WH; n =18) immediately in a slatted floor shed and offered grass silage *ad libitum* and supplementary concentrates or ii) remained at pasture for a further 35 days before housing as described (WP; n = 18). Blood samples were taken by jugular venipuncture on days (d) 0, 2, 7, 14, 21, 28, 35, 37, 42, 49 and 56. Blood evacuated into K_3-EDTA tubes was analysed for leukocyte differentials using an ADVIA 2120 haematology analyser (Siemens, UK). Blood evacuated into sodium citrate and heparin tubes was centrifuged at 3000 × g at 8°C for 10 min, and plasma was harvested and frozen at -20°C until assayed using commercial kits for fibrinogen (Roche-Boerhinger, Germany) and haptoglobin (Tridelta, Ireland) concentrations, respectively. Data were analysed as repeated measures using PROC MIXED of SAS. The model included fixed effects of time of sampling (S), treatment (T), and the possible two-way interaction (S × T).

Table 61: Treatment means (± S.E.M.) for blood cell types and APPs in WH and WP calves

		Day (d)												P level		
		0	2	7	14	21	28	35	37	42	49	56		S	T	S x T
Leu[1]	WH	13.2	13.2	11.7^{b,y}	10.2^{b,y}	10.7^{b,y}	10.8^{b,y}	10.5^{b,y}	10.6^{b,y}	10.2^{b}	10.7^{b}	11.4^{b}	F value	***	†	***
	WP	12.5	14.0^{b}	13.7^{b,x}	13.2^{a,x}	13.8^{b,x}	12.9^{x}	12.8^{x}	12.5^{x}	11.0^{c}	11.1^{c}	11.5^{c}	SEM	0.42	0.45	0.61
Neu[1]	WH	3.7^{x}	4.1	3.1	2.5^{b}	2.4^{b,y}	2.3^{b}	2.2^{b,y}	2.1^{b,y}	1.8^{b}	1.7^{b}	2.3^{b}	F value	***	†	***
	WP	2.7^{y}	3.5^{b}	3.8^{b}	3.1	3.8^{b,x}	2.5	3.1^{x}	3.8^{b,x}	2.3^{c}	2.3^{c}	2.6^{x}	SEM	1.29	1.04	1.82
Lym[1]	WH	8.4	7.9^{y}	7.6^{b,y}	7.4^{b,y}	7.5^{b,y}	7.6^{b,y}	7.6^{b}	7.8^{a}	7.7	8.4	8.4	F value	**	NS	***
	WP	8.6	9.3^{b,x}	9.0^{x}	9.0^{x}	9.0^{x}	9.1^{x}	8.6	7.8^{b,c}	7.8^{b,c}	7.9	8.2	SEM	1.38	1.17	1.96
Fib[2]	WH	369	456^{b}	560^{b,x}	499^{b,y}	500^{b}	442	425^{y}	375	439^{b}	433	335	F value	***	NS	*
	WP	384	461^{b}	467^{b,y}	545^{b,x}	475^{b}	533^{b}	518^{b,x}	401^{c}	410^{c}	454^{c}	353^{b,c}	SEM	18.8	13.0	26.7
Hap[3]	WH	35.9	54.9^{b}	63.6^{b}	65.4^{b}	53.4^{b}	53.8^{b,y}	51.2^{y}	54.8^{b}	62.7^{b}	49.5	46.0	F value	***	NS	NS
	WP	40.8	45.1^{b}	65.1^{b}	80.7^{b}	54.8	64.9^{b,x}	72.4^{bx}	67.4^{b}	67.1^{b}	64.2^{b}	50.9	SEM	0.56	0.03	0.56

† = P< 0.1, * = P<0.05, ** = P<0.01, *** = P<0.001, NS = non-significant defined as P > 0.1, $^{a,b,\ c,d,}$ means within a row with different subscripts are significantly different (P<0.05) from; ^{a,b} pre-weaning - day 0 or ^{c,d} pre-housing - day 35, baselines. ^{x,y} denotes significant difference (P < 0.05) between treatments (WH and WP) for each variable.
[1] 10^6 cells/mL, [2] mg/dL, [3] g/L

Following abrupt weaning, leukocyte (Leu) counts were decreased ($P<0.05$) in WH calves at d 7 and remained lower ($P<0.001$) than the pre-weaning baseline (d 0) for the remainder of the experimental period (Table 61). WP calves had increased ($P<0.01$) Leu counts on d 2 to d 21 compared with the pre-weaning baseline. Following housing (d 35), WP calves had a lower ($P< 0.01$) Leu counts at d 42 and remained lower than pre-housing baseline (d 35) up to d 56. Neutrophil (Neu) counts for WH calves were decreased ($P<0.01$) at d 14 and for the remainder of the experimental period, whereas it were increased at d 2 ($P<0.05$) and d 7 ($P<0.01$) for WP calves. Post-housing, Neu counts for WP calves were lower ($P< 0.05$) at d 42 and 49 compared with the pre-housing baseline (d 35). Lymphocyte (Lym) counts were decreased ($P<0.05$) from d 7 to d 35 for WH calves. For WP calves Lym counts decreased post-housing relative to the pre-housing baseline (d 35) but returned to pre-housing baseline at d 49. Fibrinogen (Fib) concentrations were elevated ($P<0.05$) for WH and WP calves up to d 21 post-weaning. Post-housing, Fib concentrations were decreased ($P<0.01$) in WP calves for the remainder of the experimental period. Haptoglobin (Hap) concentration increased ($P<0.05$) in WH and WP calves post-weaning.

Abrupt weaning altered leukocyte populations and acute phase protein profiles in WH and WP calves. The lower circulating leukocyte count observed for WH calves may suggest that abrupt weaning can be exacerbated by housing. Social, nutritional and environmental changes associated with abrupt weaning can induce an acute stress response in beef suckler calves, and this may disrupt homeostasis, lower immunity and compromise animal health.

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Behavioural assessments of beef heifers in the presence of a human

Measures of fearfulness are often based on the assumption that the reactions of animals are consistent across test situations and are stable over time (Grignard *et al.* 2001). In animals, stable behavioural responses over time are related to personality traits, whereas inconsistent responses are dependent on specific conditions. The objective of this study was to investigate the fearfulness of heifers using four behavioural tests and to determine whether their fear responses were convergent, discriminant (condition specific) or stable.

Sixty heifers (mean initial live weight 320 (s.d. 48.8) kg), comprising 42 purebred Simmental (suckled) and 18 Simmental × Friesian-Holstein (bucket-reared) ranging from 8 to 12 months of age were used. Animal behaviour was investigated using 4 fear response tests: 1) flight test (day (d) 0 and 63); 2) docility test (d 2 and 65); 3) fear test (d 4 and 67); and 4) crush test (d 69). The flight test involved placing an animal alone in an outdoor holding area (40 m length) at the end of which, a group of similar but unfamiliar (naïve) animals were penned, and (i) recording the length of time (latency) for the single animal to locate the group of naïve animals and (ii) assessing the minimum distance that an animal permitted a human to approach before moving away (escape distance). In the docility test, animals were placed singly in a 9 m^2 pen and after 30 s a person entered the pen. The time required to move the animal into a 2.25 m^2 area (1.5 m square) located in the top left corner of the pen (30 s maximum) and the time it could be maintained in that area (30 s maximum) were recorded. The fear test (reactivity to humans) was performed using a 9 m × 4.5 m pen with 1.5 m squares marked on the floor (18 squares in total). The animal's peers were present in an adjacent pen but the animal under test was prevented from seeing them by a removable blind. Each animal was tested individually. The test was divided into four phases: (i) animal alone, no stimulus; (ii) presence of a bucket of concentrate; (iii) presence of stationary

person; (iv) stationary person and animal's pen cohorts visible (blind removed). The number of squares crossed (phases i to iv), the mean distance between the animal and the stimulus and time spent eating (phases ii to iv) were recorded. The crush test was performed during blood sampling and behavioural variables related to docility were recorded as follows: number of head and feet movements, difficulty to enter the crush and difficulty to restrain the animal (4 point scale). Data that was not normally distributed was log transformed. Flight, docility and fear test data were subjected to multivariate principal component analysis (PCA) and crush test data to multiple correspondence analysis (MCA) using SPAD 6.5 (Test & Go Group) research data mining software. Differences for a variable between each phase of the fear test were assessed using a REML methodology for repeated measurements and correlations between test repetitions were calculated with a Spearman rank test (Genstat 9th Edition, VSNI).

No difference was found between the first and second flight test for latency time, however, this test was negatively correlated with the approach distance (r= -0.35, P< 0.006). The minimum approach distance for the second flight test differed between genotypes, being shorter for the crossbreds (6.8 m, s.d. 3.9) than the purebreds (13.7 m, s.d. 7.8) (P=0.002). There was no effect (P>0.05) of genotype on any other recorded variables. The fear measures of the purebred and crossbred heifers did not differ (P>0.05) using the docility, fear or crush tests. No association was found between the flight distance for the first and second test. In the docility test, a negative correlation (r =-0.45, P<0.001) was found between the time needed to drive the animal to the corner of the pen and the duration it was maintained there. There was no difference (P>0.05) between repetitions or correlations between the first and second docility test variables. The fear test showed that animals were (i) more agitated when left alone, (ii) less mobile when they had access to concentrates or in the presence of a stationary person, and (iii) the least agitated when in visual contact with peers. The shortest mean distance between them and the stimulus (food or human) was reached when an animal could see its peers, whereas the longest was in presence of the person without seeing them (Table 62). The crush test showed that animals were more agitated when handled. However, most of the animals were docile (85% entered the crush easily; 12% were difficult to handle). The PCA and MCA showed that fear related, sociability related and docility related data of heifers were not correlated.

Table 62: Behavioural responses of heifers during the fear test

	Phase			
	i	ii	iii	iv
First test				
Nbox[1]	11.5^{a}±9.6	9.1^{b}± 5.3	8.0^{b}± 4.5	5.5^{c}± 4.3
Md[2]	-	1.8^{b}± 1.0	2.8^{a}± 1.1	1.1^{c}± 0.6
Second test				
Nbox[1]	9.8^{a}± 7.7	10.0^{a}± 4.8	6.9^{b}± 4.6	5.4^{c}± 4.5
Md[2]	-	2.7^{b}± 1.3	2.6^{b}± 1.3	1.1^{a}± 0.8

[1]Nbox; Mean number of squares crossed ± s.e.
[2]Md; mean distance from stimulus ± s.e.
[a,b,c]Within row, means with the same superscripts are not significantly different (P ≥ 0.05).

The behavioural responses of the heifers using the flight, docility, fear and crush tests were condition specific and discriminant. No convergent (correlated unrelated) data were found.

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Development of a nanoparticulate vaccine to bovine respiratory syncytial virus

Bovine respiratory syncytial virus (BRSV) is a common aetiological agent in the respiratory disease complex known as enzootic calf pneumonia. This complex affects both dairy and beef cattle and is a major problem in calf rearing, both in terms of economic loss and animal welfare. During the course of an outbreak, 80-100% of affected calves will typically test positive for BRSV, with mortality rates ranging from 3 to 10%. Synthetic peptides, based on epitopes found on the G and F surface glycoproteins of BRSV, have been shown to be immunogenic and offer protection against the virus in mice. To increase their immunogenicity *in vivo*, synthetic peptides are coupled to the carrier protein, ovalbumin.

Our interest lies in developing a nanoparticulate vehicle that will deliver vaccine antigen to the dendritic cells situated in the lymph node in a targeted and prolonged manner, ultimately resulting in the stimulation of an antigen-specific immune response. The aim of this study was to manufacture and characterize two biodegradable nanoparticles containing the carrier protein, ovalbumin. To reach the dendritic cells in the lymph nodes, the particles should be less than 200nm in diameter.

Particle1 (PLGA); The polymer, poly(lactide- co-glycolide) (PLGA), has been used extensively in medicine, primarily to make internal surgical sutures and more recently, to manufacture drug delivery vehicles. Lactic and glycolic acid are normal metabolites of mammalian cellular respiration and so the polymer is completely biocompatible. PLGA particles are robust and offer good protection to the encapsulated antigen, only releasing their payload slowly over time as the particle breaks down.

Particle2 (PLGA-CS); Chitosan is the deacetylated form of chitin, which after cellulose, is the most abundant natural polymer on the planet. Coating a PLGA particle with chitosan should change its surface charge, lending it some of chitosan's mucoadhesive properties. Chitosan is also thought to temporarily open the tight junctions between endothelial cells through its interaction with F actin, giving the particle a better chance of overcoming the mucosal barrier.

Nanoparticle Manufacture: 400ml of ovalbumin in phosphate-buffered saline (PBS)[10mg/ml] was mixed with 20mg PLGA (Resomer RG502H, Boehringer, Germany) in 4ml dichloromethane (DCM, Riedle-de Haen, Germany). The two solutions were emulsified by sonicating for 1 minute at 80 watts using an ultrasonicator (VC 50 Sonic and Materials, Danbury, USA) in an ice bath to control temperature. This primary emulsion was poured into 15ml of poly (vinyl alcohol) (PVA) [10mg/ml] for stabilisation before sonication and then magnetic stirring under a vacuum for 3 hours to allow organic solvent evaporation. The nanoparticles were centrifugated 3 times for 10 minutes at 4°C and 105,000g (Optima TLX ultracentrifuge, Beckman Coulter). The pellet was kept at 4°C until resuspension and use.

For particle2, 10mg lecithin (Sigma-Aldritch, UK) was added to the PLGA/DCM solution and chitosan (low molecular weight, Sigma-Aldritch, UK) was added at 2mg/ml to the PVA solution but was otherwise as for particle1.

Nanoparticle characterisation: Nanoparticle size and zeta potential were determined using photon correlation spectroscopy (Mastersizer 3000 HS, Malvern Instruments, UK). The average particle size and zeta potential for Particle 1 and Particle 2 are shown in Table 63. Determinations were carried out on a minimum of three independently prepared samples. Nanoparticle morphology and structure was determined by scanning electron microscopy (SEM) (Figure 34). Samples of nanoparticles were attached to a metal stub and sputter coated with gold and viewed using a Hitachi S43000 SEM.

Table 63: Particle size and zeta potential of nanoparticles

Particle Type	Particle Size (nm) ± SD*	Zeta Potential (mV) ± SD*
PLGA	220.5 ± 2.2	+ 3.2 ± 0.26
PLGA-CS	478.7 ± 5.0	+10.6 ± 0.40

*where SD indicates the standard deviation

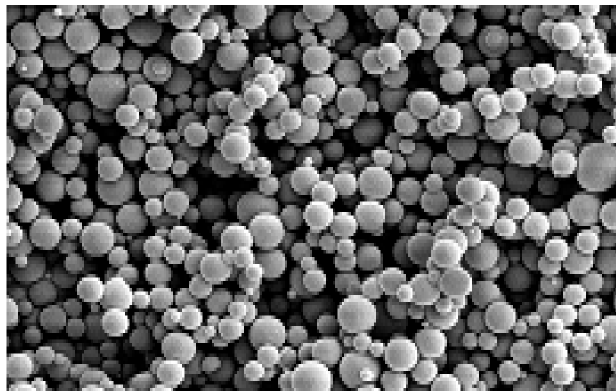

Figure 34. Chitosan-coated Nanoparticles (SEM)

The chitosan coating resulted in an increase in the average particle size and zeta potential when compared with the PLGA particle. The goal of less than 200nm for the average particle size was not achieved but should be attainable by adjusting certain formulation parameters (eg. PVA concentration, surfactant addition).

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The effect of 9-hour transportation by road on gene expression changes in circulating neutrophils of bulls

The objective of the study was to investigate changes in expression of candidate genes known to be important for neutrophil-mediated immunity and to develop a profile of possible biomarkers associated with transportation stress. Pro-inflammatory neutrophil genes, namely, those involved in apoptosis (A1 and Fas), tissue remodelling (MMP-9), margination (L-selectin), bacterial killing (BPI), and wound healing (Betaglycan) were investigated.

Six Belgian Blue × Friesian bulls, 233 ± (s.e. 3.0) kg in weight and 282 ± (s.e. 4) days of age, were transported at a stocking density of 0.85 m^2 for 9 hours on a variety of road conditions. Jugular blood was collected into acid citrate dextrose at -24, 0, 4.5, 9.75, 14.25, 24, and 48 h relative to initiation of transport. Plasma was collected to determine cortisol concentration. Neutrophils were isolated from blood, counted, and prepared for RNA extraction. cDNA synthesis was performed using Superscript II Reverse Transcriptase (Invitrogen, Carlsbad, CA) using 2µg of total RNA. The -24 h sample values were used as the calibrator according to the 2-ΔΔCt method of Livak and Schmittgen (2001). cDNA from cells collected at -24, 4.5, 9.75, 14.25, and 24 h were added to a quantitative real-time RT-PCR reaction in an Applied Biosystems 7000 or 7500 DNA sequence detection system using a SYBR Green master mix for 50 cycles. All test gene expressions were normalized against β-actin expression. The five time points for test samples were chosen to determine gene expression changes closest to times of changes in plasma cortisol and neutrophil counts relative to -24 h. The 0 h time point was omitted and the -24 h samples were used to represent true normal gene expression before the animals experienced any handling stress. All data were analyzed using the PROC MIXED procedure of SAS (version 9.1; SAS Institute, Inc.) with time relative to transportation as a fixed effect, animal and group as random effects, and weight, age, and baseline (-24 h) values for each variable included as covariates.

Plasma cortisol concentrations were elevated at 4.5 and 9.75 h compared to -24 h. Blood neutrophil counts were elevated between 4.5 and 14.25 when compared with -24 h reaching a peak of 7.22 x 10^6 at 9.75 h ($P< 0.05$). MMP-9 (Figure 35a), BPI, and L-selectin were up-regulated (P = 0.05, <0.05, and 0.05, respectively) while Fas (Figure 35b) expression was down-regulated ($P=0.02$) by transportation. The housekeeping gene β-actin was also significantly increased ($P=0.05$). The results confirm an alteration in bovine neutrophil gene expression, increases in plasma cortisol and circulating neutrophil number following transport.

Identifying changes in the expression of the inflammatory neutrophil genes involved in regulation of apoptosis, tissue remodelling, margination, and bactericidal function could begin to reveal a possible signature of imbalanced immunocompetence genes in transportation-stressed cattle. It is concluded that bovine neutrophils exhibit a hyperactive inflammatory response during transportation stress, indicating a potential risk for increased disease susceptibility following transport.

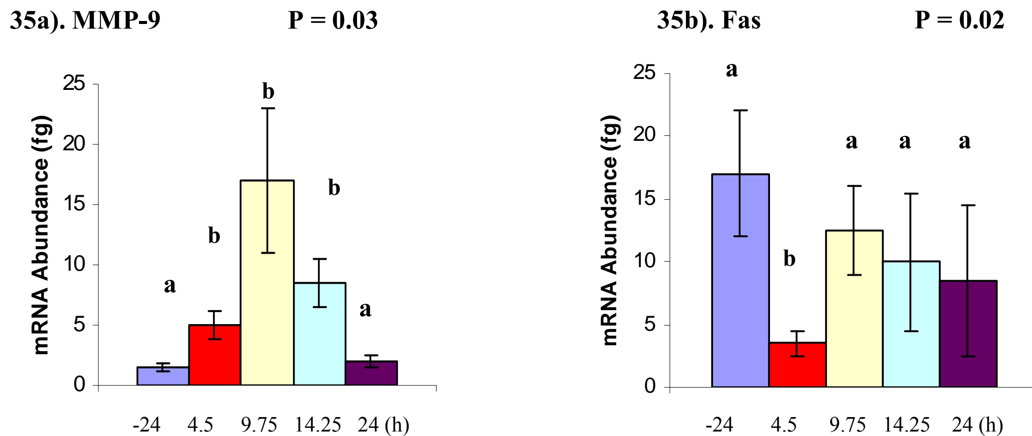

Figure 35a and 35b.

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Transportation of young beef bulls alters circulating physiological parameters that may be effective biomarkers of stress

The identification of animals with bovine respiratory disease (BRD) is usually performed by visual appraisal, which may be quite subjective. Easily measurable, but accurate, variables in the early diagnosis of BRD remain necessary. In recent years, there has been a push for the discovery of novel biomarkers to aid in the early detection, diagnosis, and therapy of complex human diseases. It is logical to hypothesize that the same approaches could be applied in livestock species for the early detection of production diseases.

The objective of the current study was to characterise a profile of physiological changes in the circulation of transported beef cattle that may act as future biomarkers of transportation stress associated with decreased production and increased disease susceptibility. The hypothesis was that transportation stress would alter numerous measures of metabolism, inflammation, and steroid hormones in the circulation and that the breed of the bulls would contribute to variability in their response to the stressor. To do so, plasma concentrations of albumin, urea, globulin, total protein, creatine kinase, β-hydroxybutyrate (βHB), haptoglobin, fibrinogen, cortisol, dehydroepiandrosterone (DHEA), calculated cortisol:DHEA ratios, testosterone, and progesterone and also circulating total leukocyte counts in transported young bulls were measured. In addition, BW and rectal temperature were recorded.

Thirty-six animals were used in this study comprising of Aberdeen Angus (n = 12), Friesian (n = 12), and Belgian Blue x Friesian bulls (n = 12), 233 ± 3.0 kg in BW and 282 ± 4 d of age at time of transportation. Bulls had *ad libitum* access to water and grass silage (*in vitro* DM digestibility = 872 g/kg), supplemented with 1.5 kg barley/soybean concentrate (CP = 104.6 g/kg DM) daily. The transportation phase of the study was conducted over 6 weeks; groups of 6 bulls were transported each week. Bulls to be transported were penned together and not commingled with

other bulls. They were transported at a stocking density of 0.85 m^2 for 9 h on a variety of road conditions, speeds, and traffic. In accordance with European Union regulation, a 45 min rest stop was observed after 4.5 h of transportation during which the animals remained on the truck. Animals were unloaded and returned to their original group pens at the end of the 9 h journey.

In the current study, transportation had effects on metabolism as demonstrated by changes ($P < 0.05$) in plasma concentrations of albumin, globulin, urea, total protein, and creatine kinase relative to pre-transportation (-24 h) values (Figure 36, Table 64). Albumin concentrations were reduced by 7% at 24 h compared with -24 h (Figure 36A). Plasma globulin was decreased by 4.5 h and remained depressed by approximately 13% throughout the time of blood collection (Figure 36B). Urea concentrations were not affected at 4.5 h, but were decreased at 48 h, 10% of the -24 h concentration (Figure 36C). In concurrence, total plasma protein concentrations were decreased by 11% at 24 h compared with -24 h (Figure 36D). In addition, a 39% decrease in plasma creatine kinase was observed at 9.75 h, followed by an increase of 221% at 24 h compared with -24 h (Figure 36E). No changes in the concentration of plasma βHB, an energy substrate, were observed (Figure 36F).

The current study also demonstrated a decrease in the plasma concentrations of the acute phase proteins haptoglobin and fibrinogen with the onset of transportation stress. Haptoglobin reached its lowest point at 4.5 h at a 53% reduction from pre-transportation values and remained depressed through 48 h (Figure 37A). Plasma fibrinogen was decreased by 44% at 14.25 h compared with -24 h, and concentrations remained depressed at all time points after -24 h (Figure 37B). The effects of time relative to transportation and breed are represented in Table 64 for these variables. In contrast with the decrease in these possible markers of inflammation, there was an increase in circulating total leukocyte counts with transportation, peaking at 9.75 h by over 21% the -24 h value (Figure 38).

Plasma cortisol was greatly elevated with the onset of transportation in the current study when compared with -24 h (Figure 39A), a 321% increase at 4.5 h compared with 13.22 ± 1.30 ng/ml at -24 h). Cortisol concentrations reached its nadir at 14.25 h at 6.99 ± 1.34 ng/ml before returning to basal concentrations at 24 and 48 h post transportation. The steroid hormone DHEA was decreased by 30% at 4.5 h (1.17 ± 0.124 ng/ml vs. 1.52 ± 0.15 ng/ml at -24 h; Figure 39B), when cortisol reached its peak. The cortisol:DHEA ratio followed the curve of plasma cortisol very closely, elevated by 528% at 4.5 h (74.62 ± 16.06 compared with 14.12 ± 2.39 at -24 h; Figure 39C).

Plasma concentrations of the gonadal steroids testosterone and progesterone were measured. Plasma testosterone was depressed with transportation stress, reaching its lowest point at 4.5 h (4.05 ± 0.20 ng/ml), 74% less than -24 h (15.41 ± 1.88 ng/ml; Figure 39D). In contrast, plasma progesterone, although present at low concentrations in bulls, was elevated at 4.5 h (0.43 ± 0.047 ng/ml), increased by 215% its -24 h concentration (0.19 ± 0.02 ng/ml; Figure 39E).

Body weight and rectal temperature were both monitored and found to be altered by transportation stress in the current study (Table 65). Body weight was decreased at 9.75 h (209.42 ± 2.25 kg) by 10% (230.78 ± 1.51 kg at -24 h) and remained decreased at 48 h after transportation (224.06 ± 1.60 kg). Rectal temperature was decreased by 48 h (38.55 ± 0.04 °C; 38.73 ± 0.06 at -24 h) but still well below a febrile state that would be indicative of an infection (> 40° C).

The breed of cattle transported appeared to have an effect ($P < 0.05$) on plasma albumin, globulin, total protein, haptoglobin, cortisol, DHEA, cortisol:DHEA ratio, and progesterone, with

tendencies toward significance ($P < 0.10$) on βHB, fibrinogen, and total leukocyte count. These results are summarized in Table 64. However, there did not appear to be any consistent trend as far as one breed responding differently to the stress of transportation than other breeds across all variables. Two profiles for variables by breed, total leukocyte count and plasma cortisol, are shown in Figure 40. The total leukocyte counts of all breeds responded similarly to stress (Figure 40A). In contrast, baseline plasma cortisol concentrations were less in Aberdeen Angus bulls than in Friesian or Belgian Blue × Friesian bulls, and although acutely increased, did not peak as highly as the other two breeds (Figure 40B).

In conclusion, we have determined that transportation stress in young beef bulls alters concentrations of physiological variables of metabolism and inflammation as well as steroid hormones in the circulation that, taken together, may be effective biomarkers of stress and disease susceptibility. Effects of breed on several physiological variables were also found to be significant but with no clear trend. Early detection of susceptible animals may aid in treatment and separation from other animals before disease increases in severity and incidence. Further validation of these potential biomarkers is required before they may be utilized in accurate diagnosis, though the current study presents a profile of possibilities.

Table 64: Effects of time relative to initiation of transportation and breed on physiological variables in plasma of transportation-stressed bulls

Physiological parameter	Effect of Time, *P*-value	Effect of Breed, *P*-value
Albumin	0.002	0.04
Globulin	< 0.001	0.04
Urea	0.006	0.40
Total Protein	< 0.001	0.007
Creatine Kinase	<0.001	0.11
βHB	0.27	0.06
Haptoglobin	<0.001	0.04
Fibrinogen	<0.001	0.05
Cortisol	<0.001	<0.001
DHEA	0.07	<0.001
Cortisol:DHEA Ratio	<0.001	0.01
Testosterone	<0.001	0.87
Progesterone	<0.001	<0.001
Total leukocyte count	0.002	0.07

Table 65: Least squares means ± SEM and effects of time and breed on BW and rectal temperature of transported bulls

Measurement	Time relative to initiation of transportation, h			Main effect, *P*	
	-24	9.75	48	Time	Breed
BW, kg	230.78 ± 1.51 ^{a}	209.42 ± 2.25 ^{c}	224.06 ± 1.60 ^{b}	<0.001	<0.001
Rectal temperature, °C	38.73 ± 0.06 ^{a}	38.86 ± 0.05 ^{a}	38.55 ± 0.04 ^{b}	<0.001	0.75

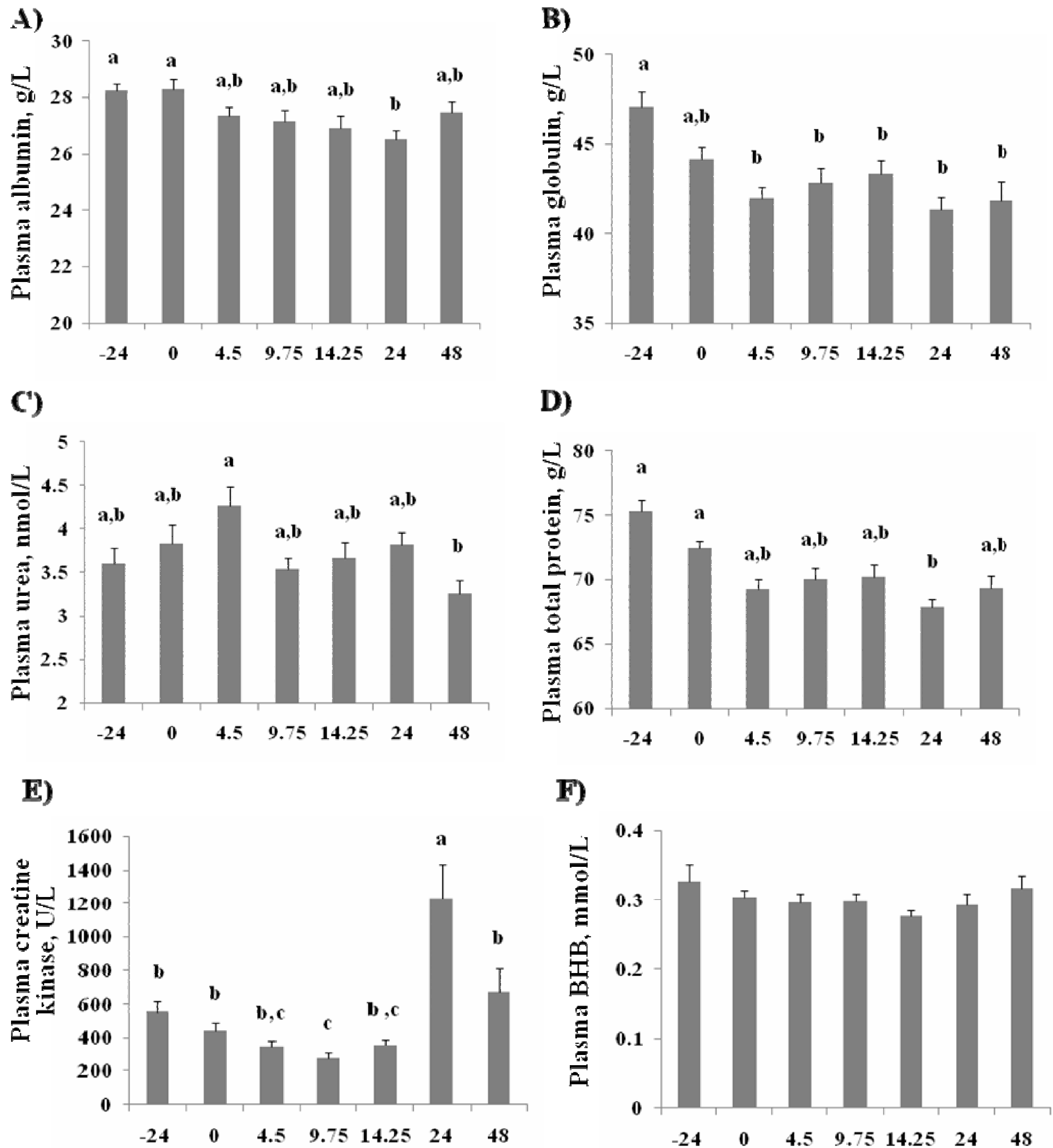

Figure 36. Profiles of measures of metabolism in the plasma of transportation-stressed bulls assayed at multiple time points relative to the initiation of a 9-h truck transportation; A) Albumin; B) Globulin; C) Urea; D) Total protein; E) Creatine kinase; F) βHB. Differences between time points are represented by different letters. *P*-values for effects of time relative to initiation of transportation at 0 h and breed are found in Table 64. For visual effect, the scales on y-axes represent maximum changes.

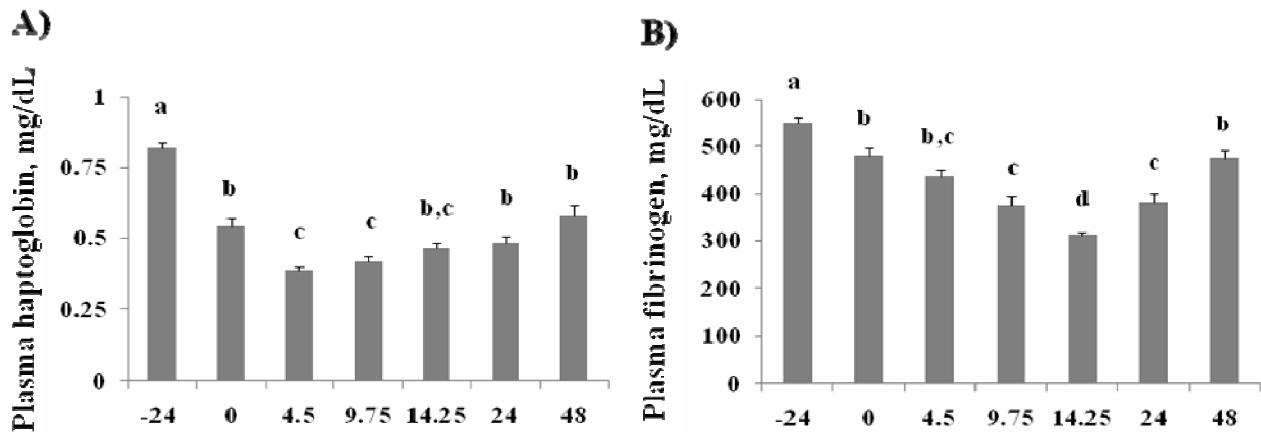

Figure 37. Profiles of acute phase proteins in the plasma of transportation-stressed bulls assayed at multiple time points relative to the initiation of 9 h truck transportation; A) Haptoglobin; B) Fibrinogen. Differences between time points are represented by different letters. The *P*-values for effects of time relative to initiation of transportation at 0 h and breed are found in Table 64.

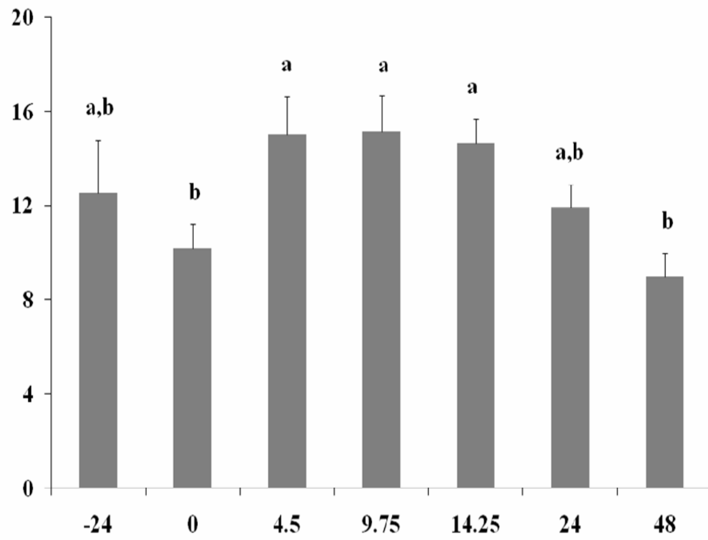

Figure 38. Total circulating leukocyte counts in transportation-stressed bulls assayed at multiple time points relative to the initiation of a 9 h truck transportation. Differences between time points are represented by different letters. The *P*-values for effects of time relative to initiation of transportation at 0 h and breed are found in Table 64.

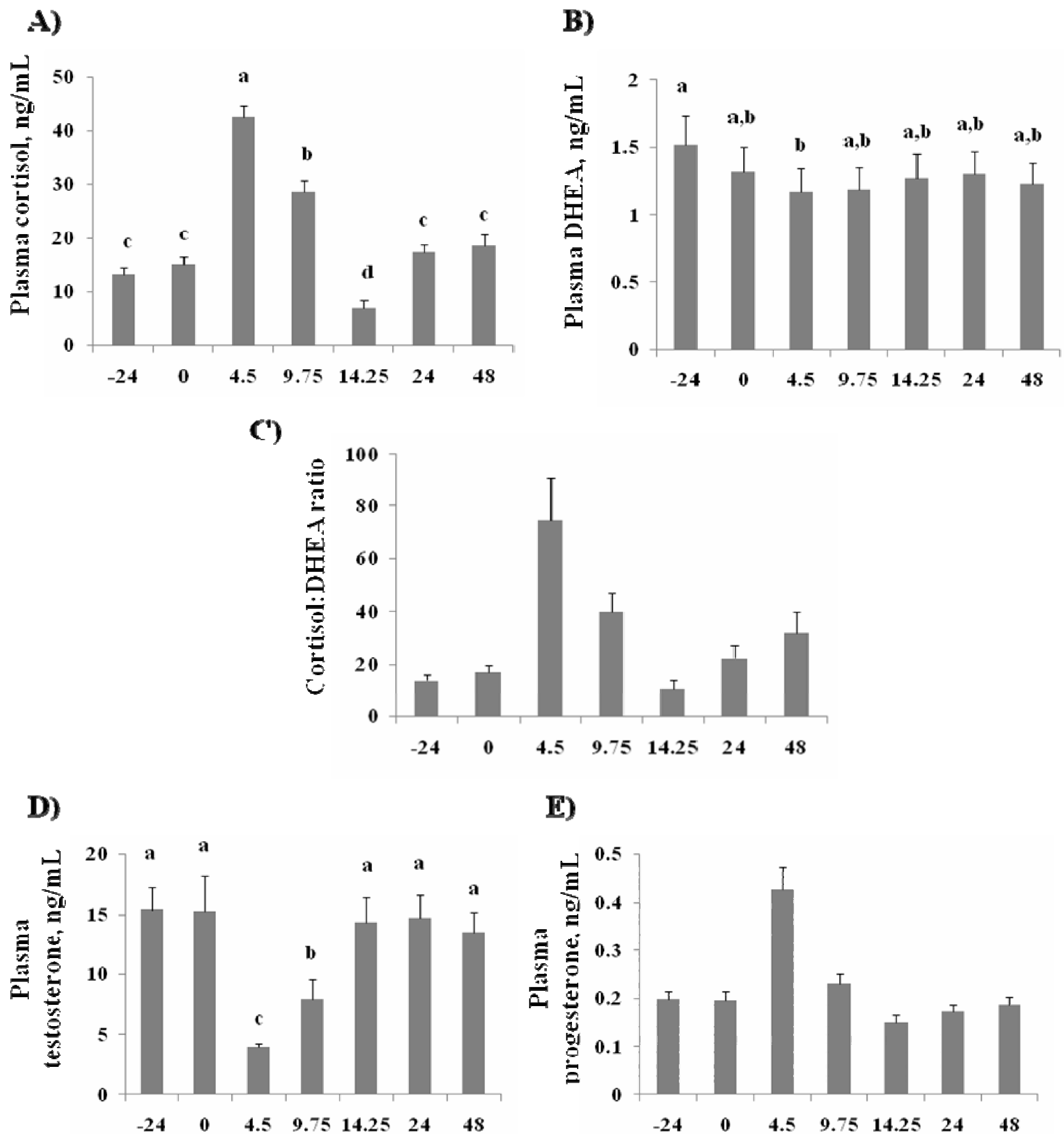

Figure 39. Profiles of plasma steroid hormones assayed in transportation-stressed bulls assayed at multiple time points relative to the initiation of a 9 h truck transportation; A) Cortisol; B) Dehydroepiandrosterone (DHEA); C) Calculated cortisol:DHEA ratios; D) Testosterone; E) Progesterone. Differences between time points are represented by different letters. The *P*-values for effects of time relative to initiation of transportation at 0 h and breed are found in Table 64.

Figure 40A

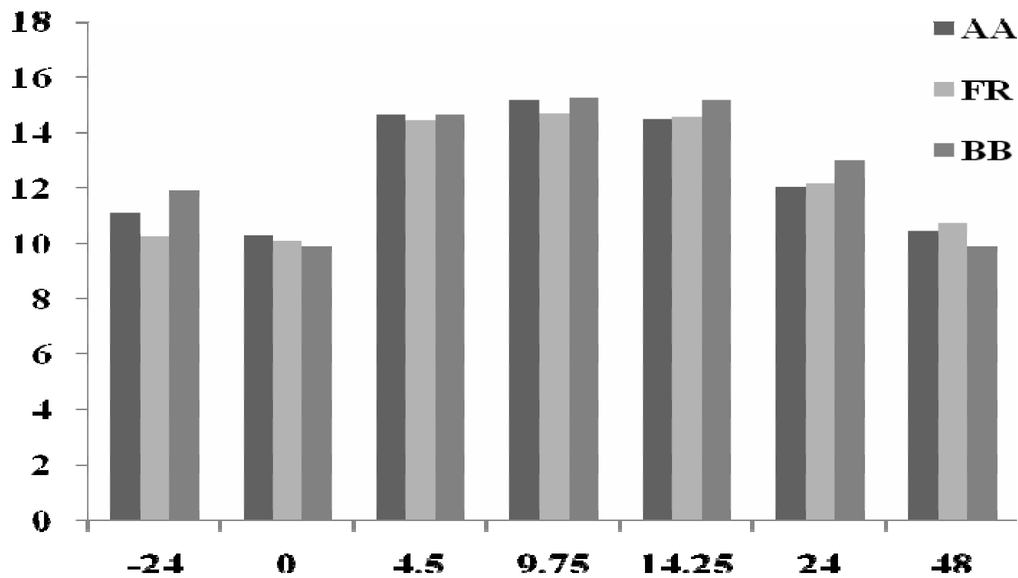

Figure 40B

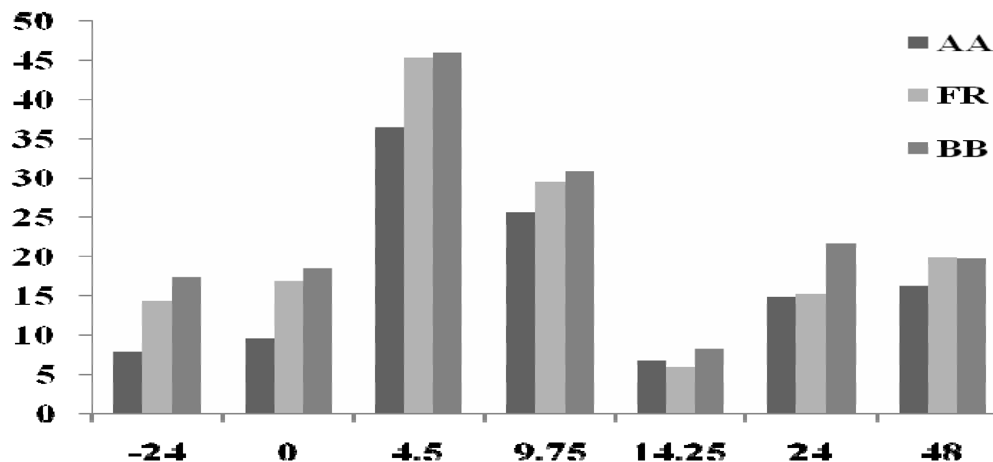

Figure 40A and B. Representative plots for variables with very little breed difference (total leukocyte counts) and for significant breed difference (plasma cortisol) in transportation-stressed bulls; A) Total leukocyte count; B) Cortisol. Standard error bars have been omitted for visual effect of differences but can be found in Figures 36 and 37A, respectively, for least squares means of all animals. Abbreviations are represented as follows: AA = Aberdeen Angus; FR = Friesian; BB = Belgian Blue × Friesian.

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