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Effect of post-weaning management practices on physiological and immunological responses of weaned beef calves

E.M. Lynch[1,2], M. McGee[3], S. Doyle[2], and B. Earley[1]†

[1]*Animal and Bioscience Research Department, Animal and Grassland Research & Innovation Centre, Teagasc, Grange, Dunsany, Co. Meath, Ireland*

[2]*Department of Biology and National Institute for Cellular Biotechnology, National University of Ireland, Maynooth, Co. Kildare, Ireland*

[3]*Livestock Systems Research Department, Animal and Grassland Research & Innovation Centre, Teagasc, Grange, Dunsany, Co. Meath, Ireland*

The objectives were: i) to investigate the physiological and immunological responses of previously grazed, abruptly weaned beef calves that were then either housed (H) and offered a diet of grass silage *ad libitum* plus concentrate or returned to familiar pasture (P) (Phase I), and ii) to examine the effect of subsequent housing (35 days post-weaning) on these responses in P calves compared with the H calves, which were acclimated to housing (Phase II). Rectal temperature was recorded and jugular blood was collected on days 0 (weaning), 2, 7, 14, 21, 28 and 35 (Phase I) and on days 0 (housing of P), 2, 7, 14, and 21 (Phase II). There was a treatment × sampling time interaction ($P<0.05$) for rectal temperature, fibrinogen concentration, total leukocyte and lymphocyte number, and phytohaemagglutinin-induced interferon-γ production during Phase I, with H calves having higher ($P<0.05$) rectal temperature and fibrinogen concentrations on day 7, lower total leukocyte and lymphocyte number on days 7 to 35 and days 2 to 28, respectively, and reduced interferon-γ production on day 7 compared with P calves. Neutrophilia ($P<0.05$) was present in P calves on days 2 and 7 post-weaning. In Phase II, total leukocyte and neutrophil numbers increased ($P<0.05$), whereas lymphocyte number declined on day 2 relative to values on day 0 of Phase II. In conclusion, deferring housing at the time of weaning resulted in a less marked stress response in beef calves compared with the traditional combined practice of weaning and simultaneous housing, however these changes were minimal

†Corresponding author: bernadette.earley@teagasc.ie

suggesting that the overall health and welfare of beef calves was not compromised by abrupt weaning and simultaneous housing.

Keywords: biomarkers; cattle; physiology; stress; weaning

Introduction

In Ireland, seasonal grass-based suckler-beef production systems typically comprise a grazing season followed by an indoor winter period. Spring-born calves have continuous access to their dams at pasture until the end of the grazing season in autumn. Calves are then weaned and are generally housed indoors for a period up to 5 months (Drennan and McGee 2009). In non-integrated systems, housing of weaned calves is preceded by transport and mixing with unfamiliar calves at livestock markets. Alterations in immune function and hormonal mediators of stress are evident in beef calves 7 days after abrupt weaning (Hickey, Drennan and Earley 2003). Bovine respiratory disease is a major animal welfare and economic concern for the beef-cattle industry, particularly with newly-weaned and feedlot cattle (Duff and Galyean 2007). Consequently, there is a need to better understand, and mitigate, stress associated with weaning, which may otherwise predispose calves to infection (Hodgson *et al.* 2005).

Weaning is a necessary husbandry practice in which nutritional, social, physical and psychological stressors are imposed on the calf. Research to date has concerned the effects of breed and age at weaning (Blanco, Casasús and Palacio 2009), and the benefit of strategies designed to mitigate stress and improve performance, such as two-stage weaning with nose-flaps (Haley, Bailey and Stookey 2005) and fence-line contact post-weaning (Price *et al.* 2003; Boland *et al.* 2008). Distress behaviours (e.g., increased vocalisation and activity) are most prominent immediately after weaning and progressively decline over the following 7 days (Price *et al.* 2003; Boland *et al.* 2008). There is, however, a paucity of literature on the effect of post-weaning management practices on the physiological responses in the calf, particularly over an extended period (> 7 days) post-weaning.

The objectives of this study were: i) to investigate the physiological and immunological responses of previously grazed, abruptly weaned beef calves that were either housed (H), and offered a new diet of grass silage *ad libitum* plus concentrates, or returned to familiar pasture (P), and ii) to examine the effect of subsequent housing (35 days post-weaning) on the responses in P calves compared with weaned calves that were acclimatized to housing (H).

Materials and Methods

All animal procedures performed in this study were conducted under experimental licence from the Irish Department of Health and Children in accordance with the Cruelty to Animals Act 1876 and the European Communities (Amendment of Cruelty to Animals Act 1876) Regulation 2002 and 2005.

Animals and experimental design

Thirty-six spring-born [mean date of birth 19 March (s.d. 25.5 days)] single-suckled (without concentrate supplementation) calves (20 males and 16 females) from first parity Limousin × Holstein-Friesian (N=18) and Simmental × Holstein-Friesian (N=18) dams by Simmental and Limousin

sires, respectively, were used. Cows and calves were rotationally grazed, in four groups that were balanced for genotype and birth date, on a predominantly perennial ryegrass (*Lolium perenne*) sward from early April until weaning on 17 October. Male calves were castrated 28 days prior to weaning.

On the day of weaning (day 0), cows and calves were moved from pasture to a handling yard and the calves were vaccinated against bovine respiratory syncytial virus, parainfluenza-3 virus and bovine viral diarrhoea (Rispoval-3; Pfizer Healthcare, Ireland). A secondary dose of Rispoval-3 vaccine was administered to all calves on day 28. Calves [mean age 212 (s.d. 25.5) days; mean weight 278.8 (s.d. 38.0) kg]were abruptly separated from their dams and assigned to either a housed treatment (H; N=18) or a pasture treatment (P; N=18). Treatments were balanced for gender (10 males and 8 females per treatment), birth date and genotype. Allocation to treatment resulted in calves from each grazing group being mixed, analogous to the social disruption experienced by weaned calves in practice. Following weaning, H calves were immediately housed in a slatted floor shed equipped with automatic drinkers; they were accommodated, loose, in 4 pens (space allowance 2.5 m^2 per calf) containing either 5 male or 4 female calves, and offered a diet of grass silage [mean dry matter digestibility (DMD) 713 (s.d. 19.5) g/kg; mean crude protein (CP) concentration 151 (s.d. 12.5) g/kg dry matter (DM)] *ad libitum* supplemented with concentrate (935 g/kg barley, 50 g/kg molasses and 15 g/kg minerals and vitamins [mean DMD 830 (s.d. 8.0) g/kg, mean CP 103 (s.d. 9.7) g/kg DM, mean neutral detergent fibre 190 (s.d. 23.7) g/kg DM] per animal daily. The concentrate allowance was increased in daily increments of 0.25 kg until an allowance of 1.0 kg per animal per day was reached. Feed analysis methodology was as described in Owens *et al.* (2009). Calves assigned to treatment P were returned immediately to a pasture of similar nutritive quality and quantity to that grazed prior to weaning, and were not supplemented with concentrates. After 35 days at pasture, P calves were housed and offered the same diet and supplementary concentrate as previously described for H calves.

Sample collection and animal measurements

Blood samples were collected by direct jugular venepuncture in two phases (Figure 1) to investigate the effect of post-weaning management. In Phase I, blood samples were collected on day 0, 2, 7, 14, 21, 28 and 35 to investigate the impact of weaning with immediate housing on physiological and immunological responses in calves. In Phase II, blood samples were collected on day 0 (= day P calves were housed), 7, 14 and 21 to examine the effect of subsequent housing on the responses in calves that had been returned to pasture post-weaning by comparison with the calves housed at weaning. Animals were moved to a handling chute for blood sampling, and mild restraint was used to obtain the blood samples. All blood samples were transported to the laboratory at ambient temperature and processed within 2 h of collection. Rectal temperature was recorded before blood sampling using a digital thermometer (Jørgen Kruuse, Marslev, Denmark). Live weight was recorded on days 0, 2, 21, 35 and 84 of the study.

Concentrations of cortisol and dehydroepiandrosterone

Blood (9 mL) collected into vacutainer tubes containing lithium heparin (Vacuette, Cruinn Diagnostics, Ireland) was used to determine the concentrations

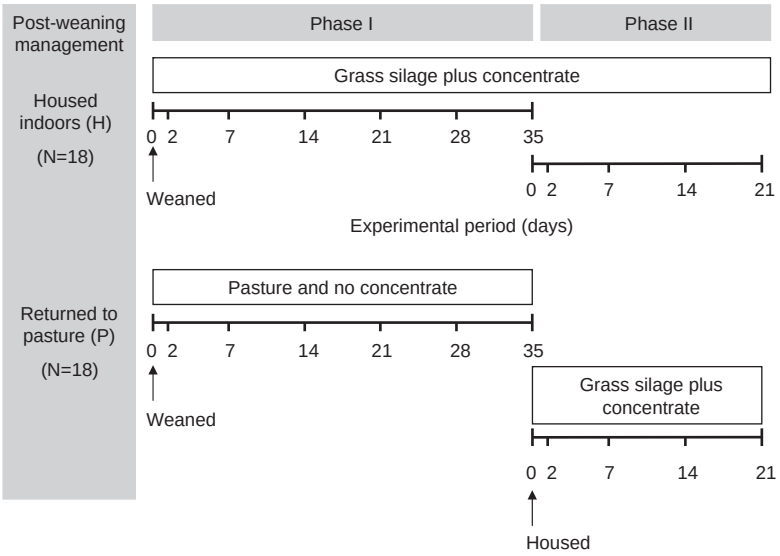

Figure 1. Management and blood sampling schedule of beef calves that were abruptly weaned and either housed (H) in a slatted floor shed and offered grass silage ad libitum plus supplementary concentrate or returned to pasture (P) without concentrate for 35 days (Phase I). Calves that were returned to pasture at weaning were housed on day 35 (day 0 of Phase II).

of cortisol and dehydroepiandrosterone (DHEA). Plasma was harvested following centrifugation (1600 × *g* at 4 °C for 15 min) and stored at –80 °C until assayed using the Correlate-EIA kits from Assay Designs (Ann Arbor, MI, USA) as per manufacturer's instructions. The mean intra-assay CV (N=11) for cortisol and DHEA was 6.4% and 4.8%, respectively; the corresponding mean inter-assay CV (N=8) was 5.7% and 4.9%. Assay sensitivity was 56.7 pg/mL for cortisol and 2.9 pg/mL for DHEA.

Haematological variables

Blood (6 mL) collected into vacutainer tubes (Vacuette, Cruinn Diagnostics, Ireland) containing K_3EDTA was used to determine total leukocyte, neutrophil, and lymphocyte numbers, red blood cell (RBC) number, haemoglobin (HGB) concentration, and haematocrit (HCT) percentage. Unclotted whole blood samples were analysed using an automatic haematology analyser (AV ADVIA 2120, Bayer Healthcare, Siemens, UK) equipped with software for bovine blood. The neutrophil:lymphocyte (N:L) ratio was calculated.

Immunological variables

Production of interferon-γ in vitro: Blood (9 mL) collected into vacutainer tubes (Vacuette, Cruinn Diagnostics, Ireland) containing lithium heparin was used to determine the *in vitro* production of interferon-γ (IFN-γ) using a whole blood culture procedure (Wood, Corner and Plackett 1990). Briefly, duplicate aliquots (1.48 mL) of blood were cultured in sterile 24-well, flat culture plates (Sarstedt Ltd., Drinagh, Wexford, Ireland) with 20 µL

of PBS (GibcoBRL, Life Technologies Ltd., Paisley, Scotland, UK) containing 1.0 mg/mL of concanavalin A (Con A) (Sigma-Aldrich, Inc., UK), phytohaemagglutinin (PHA; Sigma-Aldrich, Inc., UK) or no additive, for 24 h at 37 °C and in an atmosphere of 5% CO_2. The culture plates were then centrifuged (1600 × *g* for 20 min) at 4 °C and the supernatant harvested and frozen at –20 °C until assayed for IFN-γ using an ELISA procedure specific for bovine plasma (BOVIGAM, Biocor Animal Health, NE, USA), as previously described (Rothel *et al.* 1990). The *in vitro* production of IFN-γ, stimulated by Con A or PHA, was calculated by subtracting the absorbance, at 450 nm, for wells that received PBS alone from that for wells that received either Con A or PHA.

Acute phase proteins: Blood collected into vacutainer tubes (Vacuette, Cruinn Diagnostics, Ireland) containing lithium heparin or sodium citrate was used to determine the concentration of haptoglobin and fibrinogen, respectively. Plasma was harvested following centrifugation (1600 × *g* for 15 min) at 4 °C and stored at –80 °C until assayed. The concentration of haptoglobin was measured using an automatic analyser (spACE, Alfa Wassermann, Inc., West Caldwell, NJ, USA) and a commercial assay kit (Tridelta Development Ltd., Wicklow, Ireland) according to the manufacturer's procedure as described by Eckersall *et al.* (1999). The concentration of fibrinogen was measured using an automatic analyser (spACE, Alfa Wassermann, Inc., West Caldwell, NJ, USA) using the method described by Becker, Bartl and Wahlefed (1984).

Statistical analysis

All data were tested for normality using Proc UNIVARIATE (SAS 2003). Data that were not normally distributed (DHEA, neutrophil number, fibrinogen, Con A-, and PHA-induced IFN-γ production) were log transformed prior to statistical analysis. Data were analysed as repeated measures using the Proc MIXED (SAS 2003) with an unstructured covariance matrix and inferences were for this study in the first instance. Two datasets were analysed: i) that for Phase I (days 0 to 35), and ii) that for Phase II representing the period from the housing of animals on treatment P (days 0 to 21). The effects of sampling time, treatment, genotype and gender and all possible interactions were included in the initial model. The effects of genotype and gender were not significant and were omitted from the final model. Differences among least squares means were evaluated using a Tukey-Kramer adjustment for multiple comparisons. A probability of $P<0.05$ was selected as the level of significance.

Results

Rectal temperature and live weight

There was a treatment × time interaction ($P<0.05$) for rectal temperature during Phase I and Phase II. In Phase I rectal temperature increased ($P<0.05$) in H and P on day 2 but had returned to about initial (day 0) values on day 7 (Table 1). However, rectal temperature was greater ($P<0.05$) in H than in P on day 7, following which they did not differ ($P>0.05$). In Phase II, rectal temperature increased ($P<0.01$) in P calves and was unchanged in H calves on day 2 compared with day 0. Rectal temperature in P calves on day 7 did not differ ($P>0.5$) from that on day 0 of Phase II (Table 1).

There was no effect ($P>0.05$) of treatment on live weight at any point during the study (data not shown).

Table 1. Effect of post-weaning management on rectal temperature and the plasma concentrations of cortisol and dehydroepiandrosterone (DHEA) in weaned beef calves[§]

Variable	Treatment[†] (T)	Days post-treatment[‡] (S)							s.e.	Significance		
		0	2	7	14	21	28	35		T	S	T×S
					Phase I							
Rectal temperature (°C)	H	38.9^{a}	39.4^{b}	39.1^{a,x}	39.0^{a}	38.9^{a}	39.0^{a}	38.7^{a}	0.12		*	*
	P	39.1^{a}	39.4^{b}	38.8^{a,y}	38.7^{a}	38.5^{a}	38.8^{a}	38.7^{a}				
Cortisol (ng/mL)	H	7.2^{a,e}	9.2^{a,c,d}	9.7^{a,f}	9.9^{b,c,f}	10.4^{b,d,f}	6.0^{e,f}	6.4^{a}	0.63		***	
	P	5.5^{a}	9.1^{b,c}	8.0^{b,d}	10.1^{b,e}	9.4^{b,f}	7.0^{a,c,d}	6.3^{a,c,d,e,f}				
DHEA (ng/mL)	H	1.6	2.4	2.4	4.8	1.8	0.9	1.8	1.39		**	
	P	2.2^{a,c}	0.8^{a,c}	0.9^{a,c}	3.7^{a,c}	1.8^{a,c}	0.6^{a}	3.2^{b,c}				
					Phase II							
Rectal temperature (°C)	H	38.7^{a}	39.0^{a,x}	38.8^{a}	38.6^{a}	38.7^{a}			0.12		*	*
	P	38.7^{a}	39.5^{b,y}	38.8^{a}	38.7^{a}	38.0^{a}						
Cortisol (ng/mL)	H	6.4	6.2	6.8	5.9	6.6			0.54			
	P	6.3	6.8	6.3	6.3	6.7						
DHEA (ng/mL)	H	1.8	2.2	3.0	2.7	1.4			0.62			
	P	3.2	2.6	1.3	1.8	1.8						

[§] Data are presented as raw means; tests of significance were on transformed scale where required.

[†] H=calves weaned and housed at weaning, P=calves weaned and put to pasture for the subsequent 35 days, and then housed.

[‡] In Phase I day 0=day of weaning; in Phase II day 0=day P group were housed (=35 days post weaning).

[a,b,c,d,e,f] Means, within rows, without a superscript in common differ ($P<0.05$).

[x,y] Means, within a column for a given variable, without a superscript in common differ ($P<0.05$).

Cortisol and dehydroepiandrosterone

There was no effect of treatment or any treatment × time interaction ($P>0.05$) for the concentration of cortisol or DHEA during Phase I or Phase II (Table 1).

Leukocyte population

In Phase I, there was a treatment × time interaction ($P<0.01$) for total leukocyte, neutrophil and lymphocyte numbers, and for the N:L ratio (Table 2). Total leukocyte number was higher ($P<0.05$) in P on day 2 and day 7 compared with day 0 and lower ($P<0.05$) on days 14 to 35 in H compared with day 0 (Figure 2). Neutrophil number increased ($P<0.01$) in P on day 2 and 7, whereas it declined ($P<0.05$) in H on days 7 to 35 compared with initial (day 0) values (Table 2). Lymphocyte number in H was lower ($P<0.05$) on days 2 to 28 and higher in P on day 2 compared with values at Day 0 (Table 2). When compared with values on day 0 the NL ratio was higher on days 2 and 7 in P and lower on days 14 to 35 in H (Table 2).

In Phase II, there was a treatment × time interaction ($P<0.05$) for total leukocyte, neutrophil, and lymphocyte numbers and the N:L ratio. On days 7 to 21, total leukocyte number was lower ($P<0.05$) in P but unchanged ($P>0.05$) in H compared with day 0 (Figure 2). Neutrophil number in P increased ($P<0.05$) on day 2 and subsequently declined ($P<0.001$ on days 7 and 14) compared with day 0, whereas neutrophil number in H did not change over time ($P>0.05$) (Table 2). On day 2, lymphocyte number was lower ($P<0.05$) in P whereas it was unchanged ($P>0.05$) in H, compared with day 0. The N:L ratio increased ($P<0.05$) on day 2, and subsequently declined (days 7 to 21; $P<0.01$) in P whereas it was unchanged in H throughout Phase II (Table 2).

Haematological variables

In Phase I, there were no treatment or treatment × time interaction effects ($P>0.05$) for RBC number or the concentration of HGB. There was a treatment × time interaction ($P<0.05$) for HCT reflecting the fact that while values declined, relative to day 0, over time in both groups the decline was more abrupt in P (Table 2).

In Phase II, there was a treatment × time interaction for RBC number ($P<0.01$) and HGB concentration ($P<0.05$) due to the fact that RBC number and the concentration of HGB were lower ($P<0.05$) in H on day 14 to 21 but on days 7 to 21 in P compared with day 0 (Table 2). There was no effect ($P>0.05$) of treatment or any treatment × time interaction for HCT during Phase II.

Immunological variables

Production of IFN-γ in vitro: In Phase I, there was a treatment × time interaction ($P<0.01$) for the PHA-induced *in vitro* production of IFN-γ (Table 3), with H calves having a lower ($P<0.05$) production on day 7 compared with day 0 but no change in P calves. There was no effect of treatment or treatment × time interaction ($P>0.05$) for *in vitro* IFN-γ production induced by Con A during Phase I (Table 3).

There was no effect of treatment or any treatment × time interaction for induced IFN-γ production during Phase II (Table 3).

Acute phase proteins: In Phase I, there was a treatment × time interaction for fibrinogen ($P<0.01$) and haptoglobin ($P<0.05$) (Table 3). The concentration of fibrinogen was increased ($P<0.01$), relative to day 0, on days 2 to 21 in H and on days 2 to 35 in P. On days 7 and 35, the concentration of fibrinogen was greater ($P<0.05$) in H than in P. The concentration of haptoglobin was greater ($P<0.05$) on days 2 to 35 in H, and on days 7 to 35 in P, compared with day 0

Table 2. Effect of post-weaning management on haematological variables in weaned beef calves[§]

Variable	Treatment[†] (T)	Days post-treatment[‡] (S)							s.e.	Significance		
		0	2	7	14	21	28	35		T	S	T×S
					Phase I							
Neutrophils	H	3.9^{a}	4.1^{a}	3.1^{a,c}	2.4^{b,c}	2.5^{b,c}	2.4^{b,c}	2.3^{b,c,x}	0.29		***	***
(cells/μL)×10^{-3}	P	2.7^{a}	3.6^{b,c}	3.8^{b}	3.1^{a,c}	2.8^{a,c}	2.5^{a}	3.1^{a,c,y}				
Lymphocytes	H	8.7^{a}	8.0^{a,c}	7.7^{b,c}	7.5^{b,c}	7.7^{a,c}	7.7^{a,c}	7.8^{a,c}	0.43	*		***
(cells/μL)×10^{-3}	P	8.6	9.3	9.0	9.0	9.0	9.1	8.6				
N:L ratio[¶]	H	0.46^{a}	0.52^{a}	0.40^{a}	0.33^{b}	0.31^{b}	0.31^{b}	0.29^{b}	0.036		***	*
	P	0.33^{a}	0.40^{b}	0.43^{b}	0.36^{a}	0.32^{a}	0.31^{a}	0.38^{a}				
Erythrocytes	H	11.9^{a}	11.4^{b}	11.3^{b}	11.0^{c}	10.5^{d}	10.2^{d}	10.5^{d}	0.23		***	
(cells/μL)×10^{-6}	P	11.9^{a}	11.4^{a,c}	11.1^{b,c}	10.8^{b}	10.6^{b}	10.6^{b}	10.8^{b}				
Haemoglobin	H	144^{a}	139^{a,c}	132^{a,d}	138^{b,c,d}	131^{b}	127^{b}	131^{b}	2.4		***	
(g/L)	P	141^{a}	138^{a,c}	137^{a}	135^{b}	131^{b}	131^{b}	133^{b,c}				
Haematocrit	H	37.7^{a}	36.2^{a}	36.3^{a}	35.0^{b}	33.3^{c}	32.1^{c}	33.1^{c}	0.56		***	*
(%)	P	37.1^{a}	35.9^{a,c}	34.7^{b,c}	34.0^{b}	33.1^{b}	32.9^{d}	33.4^{b}				
					Phase II							
Neutrophils	H	2.3^{x}	2.3^{x}	1.8	1.7	2.3			0.16	*	*	*
(cells/μL)×10^{-3}	P	3.1^{a,y}	3.7^{b,y}	2.3^{c}	2.4^{c}	2.6^{a,c}						
Lymphocytes	H	7.8	7.8	7.9	8.5	8.5			0.32		*	*
(cells/μL)×10^{-3}	P	8.6^{a}	7.8^{b}	7.8^{b}	7.9^{a}	8.2^{a}						
N:L ratio[¶]	H	0.29^{x}	0.31	0.26	0.20	0.27			0.039	**	**	*
	P	0.39^{a,y}	0.50^{b}	0.29^{c}	0.29^{c}	0.33^{c}						
Erythrocytes	H	10.5^{a}	10.5^{a}	10.3^{a}	10.0^{b}	10.0^{b}			0.20		***	*
(cells/μL)×10^{-6}	P	10.8^{a}	10.7^{b}	10.0^{c}	10.3^{b}	10.0^{c}						
Haemoglobin	H	131^{a}	132^{a}	129^{a}	127^{a}	126^{a}			2.4		***	*
(g/L)	P	133^{a}	131^{a,c}	125^{b}	127^{b,c}	125^{b,c}						
Haematocrit	H	33.1^{a}	32.8^{a}	32.4^{a,c}	30.6^{b}	31.1^{b,c}			0.56		***	
(%)	P	33.4^{a}	32.9^{a,c}	32.0^{b,c}	31.0^{b,d}	30.3^{d}						

[§] Data are presented as raw means; tests of significance were on transformed scale where required.

[†,‡] See footnotes, Table 1.

[¶] Ratio of neutrophils to lymphocytes.

[a,b,c,d] Means, within rows, without a superscript in common differ ($P<0.05$).

[x,y] Means, within a column for a given variable, without a superscript in common differ ($P<0.05$).

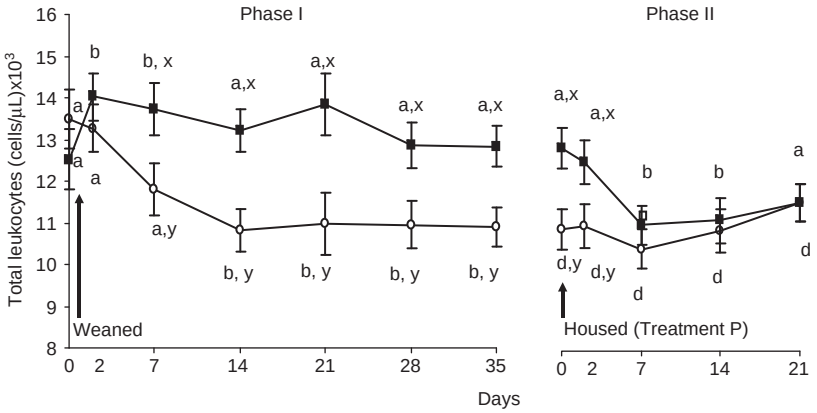

*Figure 2. Effect of post-weaning management practice on total leukocyte number in beef calves that were abruptly weaned and either housed and offered grass silage ad libitum plus supplementary concentrate (H, -○-) or returned to pasture (P, -■-) for 35 days (Phase I). Calves that were returned to pasture at weaning were housed on day 35 (day 0 of Phase II) and offered the same diet as calves in group H. The point markers 'a, b' signify differences between time points on a given line – points differ (*P<*0.05) if they have no marker in common. The point markers 'x, y' refer to differences between treatment groups at fixed time point – points differ (*P<*0.05) if the markers are different.*

(Table 3). On day 35, the concentration of haptoglobin was higher (P<0.05) in P than H.

Treatment and treatment × time interaction effects were not significant during Phase II for the concentrations of fibrinogen or haptoglobin (Table 3).

Discussion

The multifaceted nature of weaning exposes the calf to physical, physiological and psychological stressors (Weary, Jasper and Hötzel 2008). The ability of the calf to adapt to these stressors may affect subsequent health and performance (Duff and Galyean 2007). Consequently, reducing the negative impact of weaning stress is important in achieving an effective management plan for herd health. In the present study, calves from different grazing groups were mixed at weaning in order to mimic social disruption that is typically experienced by beef calves at weaning. Disruption of social groups has been shown to be stressful to cattle (Gupta *et al.* 2005) and is an important factor involved in the weaning process (Veissier and Le Neindre 1989). Elimination of milk from the diet and introduction to an entirely solid, and often novel, diet is a major adaptation required of beef calves at weaning. Moreover, low food intake is associated with stressed calves (Duff and Galyean 2007). In the present study, milk would have constituted a significant proportion of the calves' total diet at the time of weaning as milk yield of beef×dairy breed cows is relatively high at weaning (McGee, Drennan and Caffrey 2005). Although, both of the post-weaning management practices examined resulted in abrupt exclusion of milk from the calves' diet the housed calves were required to adapt to a novel diet of grass silage and concentrate, whereas the calves in the other treatment group were returned to pasture

Table 3. Effect of post-weaning management on *in vitro* production of interferon-γ induced by concanavalin A (Con A) or phytohaemagglutinin (PHA), and on plasma concentrations of haptoglobin and fibrinogen in weaned beef calves[§]

Variable	Treatment (T)	Days post-treatment (S)							s.e.	Significance		
		0	2	7	14	21	28	35		T	S	T×S
						Phase I						
IFN-γ production induced by												
Con A	H	0.86^{a}	0.71^{a}	0.34^{b}	0.55^{a}	0.97^{a}	0.55^{a}	0.68^{a}	0.164		**	
	P	0.61^{a}	0.40^{b}	0.55^{a}	0.84^{a}	0.61^{a}	0.90^{a}	0.36^{a}				
PHA	H	0.32^{a}	0.21^{a,x}	0.16^{b}	0.20^{a}	0.58^{a}	0.28^{a}	0.25^{a}	0.086		**	**
	P	0.16^{a}	0.03^{a,y}	0.12^{a}	0.18^{a}	0.25^{a}	0.62^{a}	0.29^{a}				
Haptoglobin (mg/L)	H	0.36^{a}	0.53^{b,c}	0.63^{b,d}	0.65^{b,e}	0.53^{a,c,d,e}	0.53^{b,c}	0.52^{b,c,x}	0.051	*	***	*
	P	0.41^{a}	0.45^{a,c}	0.65^{b,c,d}	0.81^{b,e}	0.55^{a,d}	0.65^{a,d,e}	0.72^{a,d,e,y}				
Fibrinogen (mg/L)	H	364^{a}	458^{b,x}	548^{c}	501^{b,d}	495^{b,d}	441^{a,b}	422^{a,b,x}	22.3		***	***
	P	384^{a}	462^{b,y}	468^{b}	545^{c,d}	475^{b}	533^{b,d}	519^{b,d,y}				
						Phase II						
IFN-γ production induced by												
Con A	H	0.68^{a}	0.31^{b}	0.29^{b}	0.50^{a}	0.42^{b}			0.145		*	
	P	0.36	0.40	0.30	0.31	0.30						
PHA	H	0.15	0.17	0.20	0.26^{x}	0.30			0.088		*	
	P	0.29	0.10	0.22	0.09^{y}	0.35						
Haptoglobin (mg/L)	H	0.52^{x}	0.55	0.59	0.48	0.44			0.054		***	
	P	0.72^{a,y}	0.67^{a}	0.67^{a}	0.64^{a}	0.51^{b}						
Fibrinogen (mg/L)	H	422^{a}	374^{a}	428^{a}	421^{a}	329^{b}			25.8		*	
	P	519^{a}	401^{b}	410^{b}	454^{b}	354^{c}						

[§] Data are presented as raw means; tests of significance were on transformed scale where required.

[†,‡] See footnotes, Table 1.

[a,b,c,d,e] Means, within rows, without a superscript in common differ ($P<0.05$).

[x,y] Means, within a column for a given variable, without a superscript in common differ ($P<0.05$).

and were not supplemented. Additionally, the indoor slatted accommodation provided a new physical environment for the housed calves. Therefore, the housed treatment may be regarded as being potentially more stressful than the pasture treatment. It is of interest that treatment had no effect on growth performance at any stage.

In the present study, calves were allowed continuous contact with their dams and unlimited access to nursing for approximately 7 months prior to weaning, whereas in a recent study calves were housed in pens adjacent to their dams for 90 or 150 days prior to weaning and were subjected to restricted suckling of two 30 minute sessions daily (Blanco *et al*. 2009). Limited contact and reduced nursing frequency from birth may result in a less intense maternal-offspring bond which may account for the smaller increase in concentration of cortisol post-weaning compared with the present study. Indoor housing in slatted-floor pens did not result in an increase in concentration of cortisol in P calves (Phase II) suggesting that the change in environment and diet were insufficient to elicit a change in this hormone. Similarly, no change in cortisol concentration was evident in beef cows when they were moved from pasture to indoor accommodation in slatted-floor pens (Lynch *et al.* 2010).

A reduction in the concentration of DHEA in combination with increased cortisol has been implicated in the stress response (Zinder and Dar 1999), reflecting the antagonistic effect of DHEA on the immunosuppressive actions of cortisol via its anti-inflammatory and immunoprotective properties (Saccó *et al.* 2002; Straub *et al.* 2002). The plasma concentration of DHEA was unchanged post-weaning, in accord with the findings of Lynch *et al.* (2010) for newly-separated (weaned) beef cows. The unaltered concentration of DHEA observed post-housing in calves that had been returned to pasture at weaning is contrary to the findings of Lynch *et al.* (2010), who reported that the concentration of DHEA declined in beef cows that were housed 35 days post-weaning. The utility of DHEA, a measure of the shift in the steroidogenic pathway from the precursor DHEA towards cortisol, as potential biomarker of management-associated stressors in the bovine requires further research.

Alterations in the components of the blood cell population are indicative of an attempt to restore homeostasis when abrupt physical conditions are encountered by an organism; thus, blood cells are very sensitive indicators of the pathophysiological responses in an animal (Jones and Allison 2007). In the present study, calves that were simultaneously weaned and housed had lower leukocyte numbers up to day 35 post-weaning compared with calves that were weaned and returned to pasture. These findings are contrary to those of Blanco *et al.* (2009) and Hickey *et al*. (2003) who reported that leukocyte number was unchanged post-weaning. However, these studies did not incorporate a change of environment in the weaning procedure, as calves were either housed from birth (Blanco *et al*. 2009) or were returned to a grazing paddock following weaning (Hickey *et al.* 2003). The changes observed in the present study may be a consequence of reduced lymphocyte and neutrophil numbers in housed calves post-weaning. A reduction in lymphocyte number has been reported in weaned calves that were housed (Hickey *et al.* 2003; Blanco *et al.* 2009) and may be due to a redistribution of lymphocytes from the peripheral circulation, in response to increased endogenous glucocorticoids and catecholamines, to tissues and organs (Nonnecke, Burton and Kehrli 1997;

Dhabhar 2002). An opposing lymphocyte profile was evident in P calves immediately post-weaning; however, this response was short-lived. While neutrophil number is often measured as a conjunct to lymphocyte number, it was found to increase in P calves post-weaning but this was not the case for H. This lack of neutrophilia in H calves is contrary to the findings of Hickey *et al.* (2003) and Blanco *et al.* (2009), who reported neutrophilia in calves that were housed post-weaning. The high neutrophil number observed for H pre-weaning likely contributes to the disparity between the present and the aforementioned studies. Following the subsequent housing of P calves, neutrophilia and lymphopenia were evident, which is in agreement with Lynch *et al.* (2010) who reported similar findings in previously grazed beef cows following housing.

Erythrocytosis, often associated with acute stress, was not evident in the present study, which concurs with the findings of Blanco *et al.* (2009). Negligible changes in the concentration of haemoglobin or in haematocrit, and a small increase in rectal temperature in Phase I and Phase II suggest that animal health was not compromised by the treatments examined in the present study.

In the present study, weaning combined with housing (a change in environment and diet) suppressed PHA-induced IFN-γ production, whereas weaning without housing did not affect IFN-γ production. These findings are contrary to Hickey *et al*. (2003) who reported that IFN-γ production was suppressed in calves that were weaned and placed in a grazing paddock. The absence of an effect of housing on *in vitro* production of IFN-γ by calves weaned for 35 days prior to housing supports the findings of Lynch *et al.* (2010) that housing did not compromise *in vitro* production of IFN-γ in beef cows.

The acute phase protein response has been used to support clinical and observational measurements in order to describe animal health and well-being (Earley and Crowe 2002; Arthington *et al.* 2003; Gånheim, Alenius and Perrson Waller 2007). The present finding that the acute phase protein response was still evident up to 35 days post-weaning conflicts with Arthington *et al.* (2008), who reported that the haptoglobin increase observed in beef calves following weaning had disappeared by day 8. Increased concentrations of fibrinogen post-weaning have been reported by others (Hickey *et al.* 2003; Qui *et al.* 2007; Blanco *et al.* 2009). The greater increase in fibrinogen concentration found in H than P calves in Phase I suggests that deferral of housing at weaning, rather than imposing both practices simultaneously, is less likely to elicit a prolonged acute phase protein response. Moreover, the absence of an acute phase protein response following housing of P calves, suggests that housing alone is not sufficient to elicit this response. The main findings of this study indicate that, compared with the traditional post-weaning management practice of weaning and simultaneous housing, deferring housing and change to a novel solid diet for 35 days post-weaning results in a less marked stress response in calves. However, the observed changes were relatively minor, suggesting that the overall health and welfare of beef calves is not compromised by abrupt weaning and simultaneous housing. This is supported by the absence of a change in cortisol and DHEA concentrations. The measurements on calves that were not housed until 35 days post weaning suggest that housing can potentially elicit a stress response but this response is short-lived and has no negative consequences for the calf.

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