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Biomarker responses to weaning stress in beef calves

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ABSTRACT

The study objective was to investigate the physiological effects of weaning on beef calves and identify a panel of blood-based welfare biomarkers. On the day (d) of weaning (d 0), 16 spring-born, single-suckled, beef bull calves that previously grazed with their dams at pasture, were assigned to one of two treatments: (1) control ($n = 8$), calves were loose-housed with their dam, (2) weaned ($n = 8$), calves were abruptly separated from their dam and loose-housed. Jugular blood was collected on d –4, 0, 1, 2, 3, 7, and 14 relative to weaning (d 0) and assayed for inflammatory and steroid variables. Total leukocyte counts were measured in whole blood. It is concluded that neutrophil number is a robust biomarker of stress and that plasma CXCL8 is a sensitive indicator of stress in weaned and control calves. In future studies, these two biomarkers should be central to the characterisation of stress responses.

1. Introduction

Weaning is a multifaceted stressor that usually involves numerous husbandry practices, including the abrupt separation of the beef calf from its dam, a nutritional adjustment to a non-milk diet and social reorganisation and, additionally, is often associated with housing (Enriquez et al., 2011; Lynch et al., 2010). Housing has been reported to alter the neutrophil and lymphocyte immunophenotype of calves, along with the acute phase response (Lynch et al., 2010), with a more pronounced stress response occurring in calves weaned at housing compared with those housed with their dams (Lynch et al., 2010, 2011). Alterations in calf immunity following weaning stress are of great importance as these changes are thought to be associated with increased incidence and severity of respiratory disease (Duff and Galyean, 2007).

Neutrophilia is one of the most frequently reported biomarkers of stress in cattle following weaning (Blanco et al., 2009; Lynch et al., 2011, 2012; O'Loughlin et al., 2011, 2012) and housing (Gupta et al., 2007; Hickey et al., 2003a). Gene expression of the potent neutrophil chemokine, CXCL8, was found to be up-regulated following transport (20-fold increase) (Gupta et al., 2007), castration (2-fold increase) (Pang et al., 2009) and weaning (2-fold increase) (O'Loughlin et al., 2011) and may account for distributional alterations in circulating neutrophils, serving to increase immune surveillance (Jones and Allison, 2007). Stress is reported to induce a response in a number of acute phase proteins (APP), including haptoglobin and serum amyloid A (SAA), with increased concentrations of APP demonstrated in calves following abrupt weaning (Hickey et al., 2003b; Martins et al., 2012) and housing (Alsemgeest et al., 1995). Stress associated with weaning has been linked to immunosuppression and greater incidences of bovine respiratory disease (BRD), mortality and related costs in newly weaned cattle entering feedlots (Hartland et al., 1991). The objective of the present study was to characterise changes in specific blood variables in beef calves subjected to either weaning and separation from their dams, or of housing with their dams.

In the present study we also hypothesised that plasma CXCL8 (formerly known as IL-8) protein levels would increase concurrently with blood neutrophils. The identification and characterisation of novel, specific biomarkers and biomarker profiles of patho-physiological states will be an important step towards the early detection of disease susceptible animals.

2. Methods

2.1. Care and use of animals

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.

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2.2. Animal model

Sixteen clinically healthy, spring-born, single-suckled, Blonde d'Aquitaine sired beef crossbred bull calves were used in this study. Prior to housing, cows and their calves were rotationally grazed on a predominantly perennial ryegrass (*Lolium* perenne) based sward from early April until housing on the 9th November. Calves were immunised 28 days prior to weaning against bovine respiratory syncytial virus (BRSV) and infectious bovine rhinotracheitis (IBR) virus using *Rispoval-3* and *Rispoval-IBR* vaccines (Pfizer Animal Health, Co. Cork, Ireland), respectively. On the day (d) of weaning (d 0), calves were moved to a handling yard and assigned to one of two treatments: (1) control ($n = 8$; mean weight (s.d.) 292.0 (36.5) kg; mean age (s.d.) 228 (22.1) days), these animals were housed with their dam, (2) weaned ($n = 8$; mean weight (s.d.) 296.5 (59.5) kg; mean age (s.d.) 242 (32.8) days), these animals were abruptly separated from their dam and housed. Pens were equipped with automatic water drinkers and the calves were offered a new diet of grass silage and had free access to concentrates.

2.3. Rectal temperature measurements

Rectal body temperature was monitored on d –4, 0, 1, 2, 3, 7 and 14 while calves were waiting in the holding chute just prior to blood sample collection using a digital thermometer (Jorgen Kruuse A/S; model VT-801 BWC Lot No 0701, Marslev, Denmark).

2.4. Sample collection

Calves were blood sampled via jugular venipuncture on d –4, 0, 1, 2, 3, 7, and 14 relative to weaning (d 0). For this procedure, they were moved gently to a holding pen with a squeeze chute facility and were blood sampled with minimal restraint. Blood sampling was carried out by the same experienced operator on each occasion and the time taken to collect the blood samples was less than 60 s/calf. Blood samples were collected into 1×6 ml spray-dried K_3ethylenediaminetetraacetic acid (K_3EDTA) coated tube (Greiner Vacuette, Cat.-No.: 456038, Cruinn Diagnostics, Ireland) for haematological analysis and 1×9 ml spray-dried lithium heparin coated tubes (Greiner Vacuette Cat.-No.: 455084, Cruinn Diagnostics, Ireland) for cortisol, acute phase protein and CXCL8 analysis.

2.5. Haematology variables

Unclotted whole K_3EDTA blood samples were analysed using an ADVIA haematology analyser (AV ADVIA 2120, Bayer Healthcare, Siemens, UK) equipped with software for bovine blood. Total leukocyte, neutrophil, lymphocyte, eosinophil and monocyte percentage, red blood cell (RBC) number, haemoglobin (HGB), mean cell haemoglobin concentration (MCV), mean corpuscular volume (MCV), haematocrit (HCT) percentage and platelet (PLT) number were measured. The neutrophil:lymphocyte (N:L) ratio was also calculated.

2.6. Acute phase proteins, cortisol and CXCL8

Plasma was harvested from the lithium heparin anti-coagulated blood tubes following centrifugation at $1600 \times g$ at 4 °C for 15 min and stored at –80 °C until analysis. The concentration of plasma haptoglobin was measured using an automatic analyser (spACE, Alfa Wassermann, Inc., West Caldwell, NJ, USA) and commercial assay kit (Tridelta Development Ltd., Wicklow, Ireland) and serum amyloid A using the SAA ELISA kit (Phase Range SAA ELISA kit, Tridelta Development Ltd., Co. Kildare, Ireland). The intra and inter assay CV for haptoglobin were 6.3% and 4.1%, respectively. SAA had an intra assay CV of 5%. Cortisol was assayed using the Correlate-EIA kit from Assay Designs (Ann Arbor, MI, USA) according to the manufacturer's instructions. Plasma CXCL8 was quantified using the Quantikine IL-8 Immunoassay (R&D Systems Europe, Ltd., Abingdon, UK) according to the manufacturer's instructions. CXCL8 had an intra and inter assay CV of 7.8% and 11.6%, respectively.

2.7. Statistical analysis

Haematological, acute phase protein, CXCL8 and cortisol data were tested for normality using PROC UNIVARIATE and the Shapiro–Wilk test and, values that were not normally distributed were log transformed prior to statistical analyses. Haematological, physiological and rectal temperature data were analysed as repeated measures using the PROC MIXED procedure of SAS (Version 9.1, SAS Institute, Cary, NC). The first sample (d –4; sample 1) was used as the baseline covariate in the statistical analysis of the data. Animal was the experimental unit and was specified as a repeated measures effect, and the dependence within animal was modelled using an unstructured covariance structure. Data subject to transformation were used to calculate *P*-values. The corresponding least squares means (Lsmeans) and SE of the non-transformed data are presented to facilitate interpretation of results. Differences between treatments were determined using the Tukey–Kramer test for multiple comparisons. Lsmeans were considered significantly different at the $P < 0.05$ probability level.

3. Results

3.1. Rectal body temperature

There was no treatment × sampling time interaction ($P > 0.05$) for rectal body temperature. Rectal temperature increased ($P < 0.01$) in both control and weaned calves (Table 1). Rectal temperature remained elevated from baseline to d 7 and was greater overall in weaned calves compared to control calves ($P < 0.03$).

Table 1
Effect of weaning induced stress at housing on rectal body temperature (°C) in weaned beef calves. The values are expressed as least squares means (Lsmeans) ± s.e.

Variable		Days post weaning						*P*-values		
		0	1	2	3	7	14	T	S	T × S
Rectal body temperature	C	38.0 ± 0.21	38.8^{b} ± 0.21	$38.7^{b,x}$ ± 0.07	38.8^{b} ± 0.12	38.6^{b} ± 0.09	38.7^{a} ± 0.11	*	*	NS
	W	38.0 ± 0.21	38.8^{b} ± 0.21	$38.7^{b,x}$ ± 0.07	38.8^{b} ± 0.12	38.6^{b} ± 0.09	38.7^{a} ± 0.11			

T, treatment; S, sampling time; T × S, treatment × sampling time interaction; NS, not significant ($P > 0.05$).
[a,b,c] Within rows, Lsmeans differ from pre-weaning baseline by $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively.
[x,y] Between rows, Lsmeans differ by $P < 0.05$.
* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.