AN EXAMINATION OF SOME PHYSIOLOGICAL VARIABLES FOR ASSESSING THE WELFARE OF BEEF CATTLE IN FEEDLOTS

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Abstract *Animal Welfare 2002, 11*: 305-316

Some physiological variables which could aid in assessing the welfare of beef cattle in feedlots were screened in this exploratory study. In two experiments, each of 42 days duration, the physiological responses of Bos taurus *steers to three treatments were investigated: pasture (rotation between 1.5 hectare paddocks); a feedlot yard stocked at 12.0 m^2 per head with a dry, firm pen surface; and a 'high-density' feedlot yard stocked at 6.0 m^2 per head with a wet and muddy pen surface. Fourteen steers were used per group per experiment. Relative adrenal mass in both feedlot groups was 8–10% higher than in the pasture group, and this finding was supported by morphological measurements of the adrenal glands. Out of 17 immune variables examined, only serum IgA and the T-cell lymphocytes subpopulation WC+1 showed consistent differences between the feedlot and pasture groups. Interestingly, no differences were observed between the two feedlot treatments. It was concluded that although there may have been some disruption of epithelial/mucosal immunity, more support was required from other immune variables before it could be stated that the immune system was depressed and that pre-pathological states existed in the feedlot groups. However, measures of relative adrenal weight, adrenal index, serum IgA and WC1+ lymphocytes are good candidates for use in future welfare investigations of feedlot cattle.*

Keywords: *animal welfare, cattle, feedlot, physiological indices*

Introduction

The issue of animal welfare is of increasing importance in agricultural industries, particularly those based on the intensive management of livestock. Although there have been investigations into the welfare status of poultry and pigs kept under intensive conditions, there have been few such studies for intensively managed cattle (Blackshaw & Blackshaw 1994; Jóhannesson & Sørenson 1999).

Approaches to assessing animal welfare are often based primarily on an examination of an animal's mental state (eg Dawkins 1990; Duncan 1996; Duncan & Petherick 1991; Wemelsfelder 1997), physical state (Kirkwood *et al* 1994; Broom 1996; Moberg 1996), or the human–animal interaction (Tannenbaum 1991; Sandøe & Simonsen 1992; Fraser 1995).

In this investigation, several physiological variables were screened as potential indicators of welfare. According to the conceptual framework of Moberg (1985, 1996), welfare is reduced if a combination of physiological changes, such as elevated hypothalamic–pituitary–adrenal axis (HPA) activity and depressed immune function, indicate that an animal has entered a pre-pathological state that represents an increased risk for the development of disease.

Methods

Two experiments were performed on matched groups of *Bos taurus* steers receiving three treatments: 1) pasture; 2) a feedlot yard with a stocking density of 12.0 m^2 per steer and a firm dry pen surface; and 3) a 'high-density' feedlot yard with a stocking density of 6.0 m^2 per steer, reduced feedbunk space and a wet and muddy pen surface. The wet pen surface was designed to interfere with lying behaviour of the steers and to create more competition for lying and feedbunk space. These conditions were designed to represent a poorly managed feedlot under prolonged wet conditions.

The study was approved by the Animal Care and Ethics Committee and was performed at the New South Wales Agriculture Elizabeth Macarthur Agricultural Institute in April–May (Experiment 1) and October–November (Experiment 2) of 1996.

Experimental animals

Forty-two beef steers were used in each experiment (14 steers per treatment group). The three groups were matched on the basis of age, breed and weight. In Experiment 1, the steers were 20 months of age at the start of the experiment and there were ten Angus and four Angus × Hereford animals in each treatment. The initial mean liveweights (± SE) were: pasture, 376 ± 6 kg; 1st feedlot treatment, 381 ± 6 kg; and high-density feedlot treatment, 384 ± 7 kg. In Experiment 2, Angus steers aged 21 months were used. The mean liveweights (± SE) were: pasture, 352 ± 4 kg; 1st feedlot treatment, 361 ± 3 kg; and high-density feedlot treatment, 359 ± 5 kg. All steers had been reared on pasture and were screened for a range of bacterial and viral pathogens prior to the experiments. All animals were free of disease. At the end of the trial, all animals were sold to market.

Pasture treatment

In both experiments, the cattle were rotated between four paddocks (300 × 50 m or 1.5 ha) as required. In Experiment 1, the pasture consisted of a 70:30 ratio of kikuyu and white clover, but the amount of available herbage was small and the animals were moved from one paddock to another every seven days. In Experiment 2, there was an 80:20 ratio of ryegrass to kikuyu grass with an abundance of pasture and the animals were rotated at 14 day intervals.

Feedlot treatments

A 13 × 13 m pen was used for the first feedlot treatment, which gave an available space of 12.0 m^2 per head. Available feedbunk space was 900 mm per head. These figures are within the recommended industry standards (Standing Committee on Agriculture and Resource Management 1997). The surface of the pen was similar to a typical feedlot pad, being a blue metal gravel base overlaid with packed dirt and manure. The walls of the pen were 1.5 m high and were lined with a rubber-and-canvas material. This was designed to minimise outside disturbances. A water trough was situated in one corner. The animals were fed in feedbunks twice daily using the feeding regimen described below.

A very similar but smaller (13 × 6.5 m) pen was used for the high-density feedlot treatment, giving an available space of 6.0 m^2 per head. This is well below the absolute minimum space requirement of 9.0 m^2 per head (SCARM 1997). The feedbunk space was also halved to 450 mm per head. The base of the pen was continually kept wet, initially through twice-daily watering. After seven days, the urine and faeces of the animals maintained this condition to a point where little further watering was needed.

Feeding regimen

Animals were fed at 0800h and 1600h each day. The ration was a mixture of chaff (lucerne, clover and oats) and a commercial pelleted feed. In accordance with normal feedlot practice, the animals were introduced to the mixture gradually, beginning with 60% roughage on days 1–4 and declining to 20% roughage by day 15. Standard *in vitro* feed analysis revealed that the chaff was approximately 90% dry matter, 14% crude protein and 8.3 MJ kg^{-1} metabolisable energy, whereas the pellets were 89% dry matter, 21% crude protein and 12.5 MJ kg^{-1} metabolisable energy. The amount fed initially was calculated as 3% of liveweight per day. Rejected feed was weighed and the amount offered at the subsequent feeding was adjusted accordingly.

Health and production measurements

Measurements took place on the day before the experiments commenced and on days 14, 28 and 42. At 16 h prior to sampling, the cattle were yarded in treatment groups and placed on a feeding curfew. At 0600h on the day of the measurements, the animals were inspected by a veterinarian and weighed. Immediately after weighing, cattle were restrained in a crush and blood and saliva samples were collected for immunological assays. Sampling of all animals was completed by 0900h. At the end of each experiment, carcass weights before chilling were recorded at the abattoir.

Measurement of adrenal activity

Both adrenal glands were removed by trained staff at the abattoir, trimmed of external fat and weighed on a top-loading pan balance to the nearest 0.01 g. The relative adrenal weight was calculated from the heaviest adrenal gland weight (g) × 100 divided by carcass weight (kg). The glands were stored in 4% phosphate buffered formalin. Sections were prepared for histological examination using standard procedures (haematoxylin–eosin staining) and then examined at 40× magnification using a Nikon Optiphot binocular microscope. A calibrated graticule (1 unit = 25 µm) was used to measure the combined width of the zona fasciculata and zona reticularis, which was compared with the thickness of the zona glomerulosa to give the adrenal index (Van Rijswijk & Vorster 1995). Six readings were made on each gland from two or three different sections taken in the same plane. All adrenal measurements were made with a 'blind' numbering system to eliminate bias.

Blood samples for plasma cortisol determinations were collected in heparinised vacutainers by jugular venepuncture and the plasma was separated and stored at –20°C (Fell *et al* 1985).

Measurement of immune competence

Blood samples were collected for haematology and immunological assays from either 7 or 14 of the animals in each group on the same days as above. Ten millilitres of blood was collected in ethylene diamine tetra-acetic acid (EDTA) vacutainers for haematology and

immunoglobulin assays, and 50 ml was collected in acid citrate dextrose for cell function assays.

Haematology

Total leucocyte counts were determined for EDTA blood using a Coulter Counter (model S880). Differential counts were performed on duplicate blood smears stained with DiffQick (Bacto Laboratories, Liverpool, NSW, Australia). Cells were classified as neutrophils, eosinophils, lymphocytes or monocytes.

Cell-mediated immune response

The T-lymphocyte subpopulations CD4+, CD5+, CD8+ and WC1+ were assayed by flow cytometry: a two-colour staining reaction using monoclonal antibody to CD45 (leucocyte common antigen) was used to identify leucocytes, and then a second antibody to a panel of cell-surface markers was used (see Table 1). Immunoglobulin-isotype-specific antibodies coupled to fluorescein isothiocyanate (FITC) and phycoerythrin (PE) were used to report the staining reaction. One hundred microlitre aliquots of EDTA blood were placed in 4.5 ml polystyrene tubes (Falcon, Becton Dickinson, Lane Cove, NSW, Australia) and incubated with 50 µl of a primary antibody (Table 1) at 4°C for 20 min in the dark. Cells were washed and centrifuged twice (5 min, 350 g, 4°C) with 2 ml of phosphate buffered saline (PBS), and then incubated with 50 µl of fluorochrome-conjugated secondary antibody for 20 min at 4°C in the dark. To lyse the majority of red blood cells, 100 µl of 8% formalin in PBS was added, and then, 1 min later, 1 ml of water at 37°C was added. Tubes were incubated at 37°C for 3 min, then 2 ml of PBS (4°C) was added and the tubes centrifuged (5 min, 350 g, 4°C). Cells were washed a second time with 2 ml of PBS and centrifuged (5 min, 350 g, 4°C). Two hundred microlitres of 1% paraformaldehyde in PBS was added and the tubes kept in the dark until acquisition of data on a FACS Vantage flow cytometer (Becton Dickinson) within 24 h of staining. Data were collected from 10,000 CD45+ events (leucocytes) and later analysed with CellQuest software (Becton Dickinson). A gate set on forward-scatter versus side-scatter plots was used to identify lymphocytes in order to determine the proportion of positively stained cells.

Table 1 Monoclonal antibodies used to identify cell surface markers on leucocytes.

Monoclonal antibody	Specificity	Isotype	Dilution	Source
CC30	CD4	IgG1	1:100	Serotec
CC63	CD8	IgG2a	1:100	Serotec
19.19	WC1	IgG1	Undiluted	CAB[b]
1.28	CD45	IgG2[a]	1:5	CAB
1.11.32	CD45	IgG1	1:5	CAB

[a] Serotec, Kidlington, Oxford, UK
[b] Centre for Animal Biotechnology, Parkville, Victoria, Australia

Immunoglobulin (Ig) determinations

Serum concentrations of IgG (immunoglobulin G) and IgA were assessed by isotype-specific ELISAs as described by Anderson *et al* (1999).

Neutrophil myeloperoxidase activity

Neutrophils were isolated as described by Anderson *et al* (1999). The rate of iodination due to myeloperoxidase activity of neutrophils following phagocytosis of opsonised zymosan was assessed by the methods of Roth and Kaeberle (1981). This assay was performed on the first experiment only.

Lymphocyte proliferation assay

The incorporation of tritiated thymidine by peripheral blood lymphocytes following stimulation with concanavalin A (ConA) and phytohaemagglutinin (PHA) was assessed as described by Anderson *et al* (1999). This assay was performed on the first experiment only.

Natural killer cell assay

The natural killer (NK) cell activity was measured with a ^{51}Cr release assay as described by Anderson *et al* (1999). Canine osteosarcoma cell line D17 was infected with infectious bovine respiratory (IBR) disease virus to prepare targets for assessment of cytotoxicity (Anderson *et al* 1999). This assay was performed on the first experiment only.

Statistical analysis

The group (of 14 animals) is the experimental unit in this study. However, because of physical and economic requirements, the conditions in the second experiment were not a true replication of the first, thus the between-group–within-treatment error was confounded with the treatment-by-experiment interaction, precluding any estimate of experimental error. The strategy adopted in this study, therefore, was to use statistical tests that employed the use of among-animal–within-group variation. The purpose of these tests was to broadly indicate which indices had consistently large differences relative to the between-animal variation in both experiments, without drawing formal inferences.

Under this caveat, each of the adrenal and body weight gain variables from both experiments were analysed using univariate analyses of variance. For each of the 17 immune variables, separate univariate analyses of the final experiment response (day 42) were conducted using the initial response as a covariate. The univariate analyses comprised both separate-experiment and combined-experiment analyses (the model for the latter consisting of the effects of treatment and experiment and treatment-by-experiment interaction).

Results

Health and production

There were no signs of clinical or subclinical disease in any animals, and no disease-related lesions on any part of the carcasses were found at the abattoir. In Experiment 1, weight gain was higher for the feedlot groups (high-density feedlot, 1.62 kg day^{-1}; 1st feedlot treatment, 1.60 kg day^{-1}) than for the pasture group, in which the animals barely maintained their weight (–0.09 kg day^{-1}). In Experiment 2, there was little difference between the groups in terms of weight gain (high-density feedlot, 1.44 kg day^{-1}; 1st feedlot treatment, 1.55 kg day^{-1}; pasture, 1.30 kg day^{-1}).

Adrenal activity

There was a higher relative adrenal weight in both feedlot treatments compared to the pasture treatment for both experiments. The mean adrenal weight (± SE) of the heavier adrenal gland (experiments combined) was 9.42 ± 0.28 g for the pasture animals, 11.27 ± 0.36 g for the 1st feedlot treatment and 11.08 ± 0.48 g for the high-density feedlot group. The relative adrenal weight (experiments combined) was 4.37 ± 0.13 g per 100 kg carcass weight for pasture, 4.79 ± 0.14 for 1st feedlot treatment and 4.76 ± 0.21 for high-density feedlot animals. A small amount of nodular hyperplasia was observed in the feedlot treatment groups.

In both experiments, the adrenal index of the pasture animals was less than that of the feedlot animals. There was a large variation amongst animals in plasma cortisol concentration, and no differences were found between groups or between days in either experiment.

Immune competence

Tables 2 and 3 show the levels of the immune variables tested pre-treatment and at days 14, 28 and 42. Serum IgA concentrations were lower in the feedlot groups than in the pasture group in both experiments. The effect was apparent after 42 days in Experiment 1 and after 14 days in Experiment 2. In Experiment 1, the difference occurred as a rise at 6 weeks in the pasture group, not as a fall in the feedlot groups. In Experiment 2, the serum IgA of the feedlot groups declined in comparison to the pasture group. WC+1 lymphocytes showed a decreased prevalence in the feedlot groups in comparison to the pasture group in both experiments, as shown by analysis of covariance (Table 4). No other immunological variables showed any consistent differences between the three treatment groups. The feedlot groups tended to have a higher neutrophil percentage and a lower lymphocyte percentage than the pasture groups at certain time points. Total leucocyte count was higher in the feedlot than the pasture groups on one occasion in each experiment. Total erythrocyte count increased during the experiment in the pasture group, but not in the feedlot groups.

Table 2 **Mean (± SE) values for a range of immune variables measured at pre-treatment, 14 days, 28 days and 42 days for cattle in pasture, 1st feedlot treatment or high-density feedlot environment. n = 7 per treatment. Experiment 1.**

	Pre-treatment	14 days	28 days	42 days
Serum IgA (mg/100ml)				
Pasture	25.7 ± 3.71	32.3 ± 4.02	26.1 ± 1.97	52.4 ± 6.25
1st feedlot treatment	26.6 ± 3.24	26.5 ± 4.89	19.2 ± 2.12	21.4 ± 3.30
High-density feedlot	25.4 ± 3.30	21.6 ± 6.27	20.4 ± 3.15	25.1 ± 2.81
Serum IgG (mg/100ml)				
Pasture	2730.8 ± 414.72	3391.5 ± 594.46	3535.9 ± 527.38	3643.3± 748.26
1st feedlot treatment	5045.4 ± 611.6	3750.8 ± 580.45	3540.8 ± 560.12	3982.0 ± 765.25
High-density feedlot	3109.9 ± 499.48	3182.7 ± 631.84	4074.1 ± 570.21	4216.9 ± 613.61
CD4+ T-lymphocytes (%)				
Pasture	27.6 ± 1.97	27.2 ± 1.70	32.1 ± 1.74	31.8 ± 1.88
1st feedlot treatment	22.3 ± 1.67	25.8 ± 0.75	27.0 ± 1.15	27.7 ± 1.27
High-density feedlot	25.4 ± 2.39	29.3 ± 1.83	30.2 ± 1.73	33.3 ± 1.82
CD8+ T-lymphocytes (%)				
Pasture	9.9 ± 1.19	12.4 ± 1.02	12.6 ± 1.03	13.3 ± 0.66
1st feedlot treatment	8.2 ± 0.51	14.1 ± 0.94	13.1 ± 0.77	14.9 ± 0.96
High-density feedlot	8.6 ± 0.93	10.6 ± 0.63	12.4 ± 0.95	13.1 ± 0.91
CD 4:8 ratio				
Pasture	3.0 ± 0.36	2.3 ± 0.19	2.7 ± 0.27	2.4 ± 0.14
1st feedlot treatment	2.7 ± 0.20	1.9 ± 0.14	2.1 ± 0.17	2.0 ± 0.26
High-density feedlot	3.0 ± 0.28	2.8 ± 0.22	2.5 ± 0.22	2.6 ± 0.20
CD5+ T-lymphocytes (%)				
Pasture	61.5 ± 3.54	66.9 ± 1.49	73.4 ± 1.66	54.0 ± 1.16
1st feedlot treatment	54.4 ± 3.55	72.9 ± 1.55	71.2 ± 2.33	51.7 ± 0.82
High-density feedlot	64.3 ± 3.52	64.7 ± 0.85	72.1 ± 1.06	55.0 ± 1.43
WC1+ T-lymphocytes (%)				
Pasture	18.3 ± 1.60	15.2 ± 1.81	19.5 ± 0.96	19.5 ± 1.31
1st feedlot treatment	20.6 ± 1.36	19.3 ± 1.91	19.5 ± 1.81	18.4 ± 2.02
High-density feedlot	23.3 ± 3.04	16.2 ± 1.88	17.7 ± 1.42	15.9 ± 1.84
Natural killer cell assay (% cytotoxicity)				
Pasture	47.8 ± 2.29	35.2 ± 3.55	57.0 ± 2.88	40.4 ± 3.09
1st feedlot treatment	51.4 ± 2.06	34.0 ± 1.73	58.1 ± 2.16	51.5 ± 4.20
High-density feedlot	48.4 ± 3.10	35.3 ± 2.78	53.59 ± 2.25	36.2 ± 2.06

Neutrophil myeloperoxidase assay (iodination rate)				
Pasture	26.1 ± 4.87	22.6 ± 6.73	17.5 ± 5.42	37.6 ± 6.44
1st feedlot treatment	25.9 ± 3.91	33.0 ± 4.39	21.4 ± 1.67	35.3 ± 1.87
High-density feedlot	24.6 ± 6.10	30.0 ± 6.99	16.7 ± 2.63	25.6 ± 2.25
Total leucocyte count (10^9/L)				
Pasture	6.6 ± 0.54	6.9 ± 0.50	5.9 ± 0.53	6.1 ± 0.55
1st feedlot treatment	7.3 ± 0.52	7.3 ± 0.55	8.3 ± 0.50	8.3 ± 0.54
High-density feedlot	6.6 ± 0.37	7.1 ± 0.52	7.8 ± 0.30	6.6 ± 0.47
Lymphocytes (%)				
Pasture	62.0 ± 4.24	65.7 ± 2.47	66.1 ± 2.25	70.9 ± 1.00
1st feedlot treatment	63.2 ± 4.72	64.5 ± 3.17	56.5 ± 2.43	57.8 ± 2.73
High-density feedlot	62.3 ± 1.68	58.7 ± 4.53	56.0 ± 1.89	64.3 ± 2.64
Monocytes (%)				
Pasture	10.7 ± 1.49	5.4 ± 0.25	9.2 ± 1.07	6.8 ± 0.54
1st feedlot treatment	8.8 ± 1.21	6.6 ± 0.27	12.1 ± 0.70	8.9 ± 0.63
High-density feedlot	9.0 ± 0.72	8.3 ± 0.68	11.9 ± 0.93	9.3 ± 0.98
Neutrophils (%)				
Pasture	23.3 ± 4.47	24.6 ± 2.78	18.0 ± 2.90	15.1 ± 1.42
1st feedlot treatment	24.2 ± 4.13	25.5 ± 2.97	24.7 ± 2.17	26.2 ± 3.15
High-density feedlot	25.8 ± 1.49	30.2 ± 4.20	29.0 ± 1.90	21.5 ± 2.59
Eosinophils (%)				
Pasture	3.9 ± 1.56	4.3 ± 1.32	6.7 ± 2.32	7.2 ± 0.79
1st feedlot treatment	3.8 ± 1.11	3.3 ± 0.82	6.7 ± 1.0	7.2 ± 1.44
High-density feedlot	2.9 ± 0.88	2.7 ± 0.43	3.2 ± 0.63	4.8 ± 0.68
Lymphocyte proliferation assay Unstimulated (net count per million)				
Pasture	1178 ± 307.6	4439 ± 713.6	3840 ± 700.6	376 ± 47.6
1st feedlot treatment	3328 ± 1387.0	2108 ± 655.8	4813 ± 967.5	601 ± 165.7
High-density feedlot	1153 ± 221.5	1152 ± 158.5	1070 ± 194.9	231 ± 13.5
Lymphocyte proliferation assay Concanavalin A (net count per million)				
Pasture	124600 ± 7150	96000 ± 6167	43270 ± 5668	29080 ± 4733
1st feedlot treatment	114000 ± 14220	75800 ± 6465	41520 ± 4666	38400 ± 10660
High-density feedlot	135800 ± 15690	95460 ± 11050	98820 ± 10540	28860 ± 9865
Lymphocyte proliferation assay Phytohaemagglutinin (net count per million)				
Pasture	87840 ± 6695	102600 ± 9102	106600 ± 10380	48360 ± 8179
1st feedlot treatment	96780 ± 11630	91600 ± 9203	92380 ± 2616	49330 ± 6641
High-density feedlot	100100 ± 15230	113100 ± 9612	110200 ± 11740	53450 ± 6462

Table 3 **Mean (± SE) values for a range of immune variables measured at pre-treatment, 14 days, 28 days and 42 days for cattle in pasture, 1st feedlot treatment or high-density feedlot environment. n = 14 per treatment. Experiment 2.**

	Pre-treatment	**14 days**	**28 days**	**42 days**
Serum IgA (mg/100ml)				
Pasture	53.2 ± 8.10	51.5 ± 5.62	38.4 ± 3.32	49.4 ± 4.91
1st feedlot treatment	43.1 ± 5.85	37.8 ± 5.74	28.6 ± 2.99	31.8 ± 3.54
High-density feedlot	41.2 ± 4.25	27.4 ± 4.86	23.1 ± 3.82	23.7 ± 2.91
Serum IgG (mg/100ml)				
Pasture	1597.2 ± 302.03	1823.7 ± 316.79	4134.9 ± 498.43	4439.7 ± 652.99
1st feedlot treatment	3571.7 ± 766.86	2143.4 ± 597.29	2591.2 ± 385.08	3322.2 ± 579.90
High-density feedlot	2545.6 ± 601.02	1549.9 ± 228.01	3459.0 ± 515.56	3315.4 ± 567.27
CD4+ T-lymphocytes (%)				
Pasture	27.1 ± 1.58	25.7 ± 1.30	22.1 ± 1.58	25.6 ± 1.60
1st feedlot treatment	27.5 ± 1.37	23.8 ± 1.42	26.0 ± 1.58	25.0 ± 1.70
High-density feedlot	26.2 ± 0.94	26.1 ± 1.42	26.0 ± 1.07	26.4 ± 1.17
CD8+ T-lymphocytes (%)				
Pasture	14.7 ± 0.43	13.8 ± 0.59	11.0 ± 0.60	12.5 ± 1.07
1st feedlot treatment	13.6 ± 0.81	12.2 ± 0.55	10.2 ± 0.47	11.8 ± 0.76
High-density feedlot	14.5 ± 0.85	13.1 ± 0.93	12.3 ± 1.03	14.1 ± 0.89

CD 4:8 ratio				
Pasture	1.9 ± 0.11	1.9 ± 0.10	2.1 ± 0.17	2.2 ± 0.15
1st feedlot treatment	2.1 ± 0.14	2.0 ± 0.15	2.7 ± 0.24	2.2 ± 0.17
High-density feedlot	1.9 ± 0.09	2.0 ± 0.13	2.2 ± 0.15	1.9 ± 0.11
CD5+ T-lymphocytes (%)				
Pasture	67.7 ± 2.24	58.0 ± 2.48	45.2 ± 2.41	40.9 ± 2.16
1st feedlot treatment	54.2 ± 1.47	52.9 ± 1.72	36.4 ± 2.64	41.2 ± 1.90
High-density feedlot	66.3 ± 2.53	56.0 ± 2.66	44.7 ± 2.16	49.7 ± 1.75
WC1+ T-lymphocytes (%)				
Pasture	14.2 ± 1.79	15.2 ± 1.85	15.0 ± 2.01	15.9 ± 2.45
1st feedlot treatment	12.2 ± 1.05	12.5 ± 1.01	14.2 ± 2.17	9.8 ± 0.87
High-density feedlot	13.8 ± 1.10	12.5 ± 0.95	14.3 ± 1.28	12.6 ± 1.16
Total leucocyte count (10^9/L)				
Pasture	9.1 ± 0.71	8.7 ± 0.34	9.0 ± 0.51	7.9 ± 0.21
1st feedlot treatment	9.4 ± 0.58	11.0 ± 0.80	10.0 ± 0.69	8.1 ± 0.29
High-density feedlot	9.3 ± 0.54	10.5 ± 0.72	10.3 ± 1.00	8.9 ± 0.51
Lymphocytes (%)				
Pasture	65.8 ± 2.84	49.4 ± 2.34	60.5 ± 1.98	56.8 ± 3.09
1st feedlot treatment	70.3 ± 1.85	44.3 ± 3.79	54.4 ± 2.56	62.9 ± 3.00
High-density feedlot	69.1 ± 1.54	38.6 ± 2.88	61.6 ± 1.75	58.5 ± 2.66
Monocytes (%)				
Pasture	10.4 ± 0.63	12.1 ± 1.06	11.2 ± 1.06	10.5 ± 0.54
1st feedlot treatment	10.7 ± 0.65	10.4 ± 0.69	9.4 ± 0.92	12.1 ± 0.97
High-density feedlot	11.3 ± 0.46	11.1 ± 1.43	11.3 ± 0.81	10.4 ± 1.02
Neutrophils (%)				
Pasture	16.7 ± 2.48	32.3 ± 3.16	24.4 ± 2.50	27.7 ± 3.36
1st feedlot treatment	15.5 ± 1.71	39.0 ± 4.42	33.8 ± 3.16	19.2 ± 2.73
High-density feedlot	14.9 ± 1.18	42.5 ± 4.17	24.4 ± 1.75	28.0 ± 3.23
Eosinophils (%)				
Pasture	7.1 ± 1.89	4.3 ± 0.49	3.4 ± 0.28	4.0 ± 0.57
1st feedlot treatment	3.5 ± 0.53	3.7 ± 0.95	2.3 ± 0.44	3.5 ± 0.78
High-density feedlot	4.6 ± 0.62	4.9 ± 0.59	2.6 ± 0.30	3.0 ± 0.30
Lymphocyte proliferation assay Unstimulated (net count per million)				
Pasture	1179 ± 298.0			1241 ± 179.5
1st feedlot treatment	955 ± 211.5			1160 ± 191.4
High-density feedlot	973 ± 238.8			990 ± 98.5
Lymphocyte proliferation assay Concanavalin A (net count per million)				
Pasture	41270 ± 4383			56120 ± 7420
1st feedlot treatment	40160 ± 4250			53220 ± 4856
High-density feedlot	49880 ± 8157			50580 ± 6599
Lymphocyte proliferation assay Phytohaemagglutinin (net count per million)				
Pasture	33940 ± 4742			44380 ± 8652
1st feedlot treatment	24040 ± 3592			39630 ± 6627
High-density feedlot	30140 ± 5122			25490 ± 2363

Table 4 **Analysis of covariance results for WC1+ lymphocytes for cattle placed in either pasture, 1st feedlot treatment or high-density feedlot environment for 42 days. n = 7 per treatment in Experiment 1 and n = 14 per treatment in Experiment 2.**

	Experiment 1	Experiment 2
Pasture	20.4 ± 1.62	15.2 ± 1.03
1st feedlot treatment	17.5 ± 1.69	10.8 ± 1.07
High-density feedlot	14.9 ± 1.62	12.2 ± 1.02

Discussion

The measurement of a combination of physiological and/or behavioural changes, rather than of a single marker, has been proposed by Moberg (1985, 1996) as a basis for assessing whether stressors perturb homeostatic mechanisms sufficiently to place animals at a heightened risk of developing behavioural, metabolic or immunological pathologies, or an unequivocal disease state. This state of heightened susceptibility has been termed "pre-pathological change" by Moberg (1985). In an analogous fashion, Amadori *et al* (1997) have used a panel of immunological and haematological tests to assess the welfare of dairy cattle. This multifactorial approach was adopted in the current study to ascertain the utility of various physiological measurements for assessing the welfare of feedlot cattle. To this end, the experimental design employed a crowded feedlot with a wet and muddy substratum that, it was anticipated, would alter behavioural patterns and impose a range of stressors on cattle. Behavioural patterns of cattle in all treatment groups were examined and are to be reported elsewhere.

Elevation of HPA activity manifests itself in increased secretion of the glucocorticoid, cortisol, which is produced solely by cells of the zona fasciculata in the adrenal cortex. Chronic increases in cortisol production are associated with expansion of the zona fasciculata and increased adrenal mass. Therefore, the weight of the adrenal glands relative to the body weight of the animal, and the relative size of the zona fasciculata (or adrenal index), can be used as indicators of elevated HPA activity (Appleby & Sohrabi 1978; Pliška *et al* 1985; Van Rijswijk & Vorster 1995).

Both feedlot groups in the study showed increased HPA activity, as evidenced by larger adrenal glands and an expansion of cortisol-secreting tissue. The increase in relative adrenal weight was of the magnitude 8–10% in the feedlot compared to the pasture animals, but there was no difference between the two feedlot groups. From adrenal gland data alone, it cannot be determined whether this change represents a response to chronic stress or an adaptive change to the feedlot environment. Hyperplasia of the zona fasciculata can occur in cattle in response to various stressors (Hartmann & Gunther 1974; Makumyaviri *et al* 1985), although quantitative data for comparison with the current results are scarce. The small amount of nodular hyperplasia observed in the present study can occur in domestic animals in the absence of known stressors (Appleby & Sohrabi 1978).

Two immunological variables out of the 17 measured, WC1+ lymphocytes and serum IgA, showed consistently lower values in both feedlot treatments compared to the pasture treatment, although there were no differences between the feedlot treatments. WC1+ lymphocytes are mainly located in epithelial tissues and may function as a primary line of defence at these sites. The prevalence of WC1+ lymphocytes in blood is reduced by exogenous corticosteroids (Burton & Kerhli 1996; Anderson *et al* 1999). Morrow-Tesch *et al* (1996) noted changes in the levels of these cells in cattle undergoing heat-related treatments. In sheep, Cockram *et al* (1993) found that isolation from the flock increased neutrophil numbers and reduced the numbers of some lymphocytes, including WC1+ lymphocytes. Chronic heat stress was found to impair lymphocyte proliferation in sheep (Niwano *et al* 1990). Baldwin *et al* (2000) concluded that either stress or the housing environment reduces WC1+ numbers in calves, which is in accord with the results of the current study.

The contribution of secretory IgA to mucosal immunity is well documented (see eg Drummond & Hewson-Bower 1997; Kagnoff & Kiyono 1996) and depressed levels are associated with increased risk of infection. Serum IgA has been less commonly measured in studies of stress than salivary IgA (eg Rocker *et al* 1978; Maes *et al* 1997; Pariante *et al*

1997). The link between serum IgA and secretory IgA is not necessarily a direct one. For example, Florence *et al* (1995) reported that in laying hens, levels of serum IgA did not fluctuate in direct correspondence to levels of secretory IgA. Serum IgM, but not salivary or serum IgA, was found to be depressed by administration of exogenous corticosteroids in cattle (Anderson *et al* 1999). Heat stress of cows in late pregnancy suppresses IgA transport into the colostrum (Nandone *et al* 1997), although the stress of dystocia does not depress adsorption of IgA by the newborn calf (Stott & Reinhard 1978). Together these reports from the literature on IgA and lymphocyte subpopulations illustrate the variability of the effects of stressors on the immune system. Amadori *et al* (1997) considered values that lay outside the expected range recorded in their laboratory to flag a potential compromise of welfare and health outcomes in cattle. Our approach used a comparison of values between pasture and feedlot treatments, and makes the assumption that values for animals at pasture represent the norm. However, the possibility of antigenic pressure affecting levels of serum IgA in the pasture animals needs to be considered. In this study, serum IgA levels in Experiment 1 increased in the pasture animals towards the end of the experiment. Although there were no signs of clinical illness or elevations in other immunological markers indicative of disease, the existence of an antigenic challenge (possibly parasitic in nature) as the basis of the difference in IgA levels between pasture and feedlot groups cannot be ruled out.

Investigations of this nature require large amounts of physical and monetary resources and time, and so for this study we have been restricted to an exploratory investigation. However, our results and the inconsistent nature of other published findings reviewed above are such that we could not conclude that any animals in this study were in a pre-pathological state. If extra stressors such as the social mixing of unfamiliar cattle and/or bacterial or viral pathogens had been introduced, the capacity of these steers to adapt may have been exceeded, allowing the development of pre-pathological states and heightened risk of disease.

Animal welfare implications

The data from this preliminary study show that measures of relative adrenal weight, adrenal index, serum IgA and WC1+ lymphocytes may be useful markers for future studies on the welfare of cattle. Further work on physiological indicators of welfare would benefit from greater pre-treatment sampling to establish better baseline values of immune variables for experimental subjects and perhaps by the introduction of additional stressors that are frequently associated with induction of cattle to the feedlot environment.

Acknowledgements

We thank Keith Walker and Paul Nicholls for helpful advice and acknowledge the dedicated technical assistance of F Bertus, B Anderson, L Baker, N Franklin, G Brown, G Furley, J House, H Vallance and K Van Hoff. This work was supported by funds from the Cooperative Research Centre for Cattle and Beef Quality.

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