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Animal Welfare 2008, 17: 19-26
ISSN 0962-7286

Assessment of welfare from physiological and behavioural responses of New Zealand dairy cows exposed to cold and wet conditions

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Abstract

There is a need to assess the welfare of dairy cows that live outdoors under cold and wet conditions. This study combined a number of techniques to measure stress and make an assessment of welfare in this situation. Two groups of ten non-pregnant, non-lactating Holstein Friesian cows were exposed to a week of wind and rain (WR) or housed indoors (I) with pre- and post-treatment weeks indoors in a cross-over design. Wind and rain consisted of continual air movement (7.1 kph) using fans, water sprinkling for 15 min (3.0 mm) per hour, a mean temperature of 3.4°C and wind chill of –0.3°C. Internal body temperature was recorded every ten min and behaviour for 16 h per day. Blood, faeces and infrared temperatures were sampled at 0800h each morning during treatment weeks, and three times per week during pre- and post-treatment weeks. All cows were challenged with 2 ml Leptoshield Vaccine (CSL Animal Health, Australia) subcutaneously after 3 days of cold exposure to test immune responses. During WR, cows spent a greater proportion of time standing and less time lying down and eating than during I. Infrared temperatures were lower during WR than I in both dorsal and orbital (eye) regions. There was a distinct diurnal pattern of internal body temperature which had a greater amplitude during WR than I resulting from both a lower minimum and a higher maximum. The time of the minimum was 40 min later for WR than I. The overall mean body temperature was 0.07°C higher in WR than I. There were greater increases in plasma and faecal cortisol during WR than I, respectively. Total T4 was higher during WR than I. Non-esterified fatty acid concentration was higher in the week following WR than I. Total white blood cell numbers were lower during WR than I. No treatment differences were found for creatine kinase or for tumour necrosis factor, heat shock protein 90, interleukin 6 or interferon gamma expression in response to vaccination. In conclusion, this study applied a suite of stress measures to dairy cows exposed to extreme cold and wet conditions. Together, these measures indicated activation of the stress axis, physiological and behavioural adaptations to cold and a reduction in welfare. A number of these measures could be used to assess welfare under cold conditions on farms.

Keywords: *adaptation, animal welfare, behaviour, cold, dairy cows, stress*

Introduction

Dairy cows in New Zealand live predominantly outdoors on pasture all year round. Outdoor farming allows animals to behave in a more normal manner than is possible under intensive, indoor-based situations and subsequently has a more positive welfare image (Hemsworth *et al* 1995). However, there are welfare issues under pastoral grazing systems and an obvious one is caused by exposure to the climate. Thermal discomfort is considered to be an important cause of animal stress and an animal's ability to ameliorate its effects by finding shade or shelter may be limited in some farming situations (Gregory 1995). The New Zealand climate is generally mild with mean sea level temperatures ranging from about 15 to 10°C from the north to the south, respectively and the annual range of mean monthly temperature is small, ranging from 8 to 14°C. A feature of the climate, however, is changeability, with an irregular succession of anticyclones, every six to seven days, often with cold, showery south-westerly winds between. The result is that cows may be exposed to sudden, brief periods of cold and wet weather. Heat loss in cattle is increased by wind and rain (Webster 1974; Ames & Insley 1975) which reduces the insulative properties of the hair coat. This may act to increase the relative severity of windy and wet weather conditions. Adaptation to cold in beef cattle is dependent on the duration of cold exposure and metabolic acclimation does not occur in response to intermittent cold exposure (Bergen *et al* 2001; Kennedy *et al* 2005). Short periods of cold, windy and wet weather may be difficult to adapt to and if there is no suitable shelter available, may reduce the welfare of dairy cows. As well as a direct thermal effect, there is evidence that cold exposure impairs the immune

https://doi.org/10.1017/S0962728600031948 Published online by Cambridge University Press

Table 1 Experimental design.

Week number	Group 1	Group 2
1	Indoor	Indoor
2	Wind and rain	Indoor
3	Indoor	Indoor
4	Indoor	Wind and rain
5	Indoor	Indoor

system (Kelley *et al* 1980) making animals more susceptible to infection or reducing the efficacy of vaccinations (Kehrli *et al* 1999).

To assess the welfare of dairy cows on pastoral-based systems it is necessary to detect signs of discomfort and stress for dairy cows under cold and wet conditions, preferably in a relatively non-invasive manner. Three non-invasive measures of stress used in this study were faecal cortisol levels (Morrow *et al* 2002), circadian body temperature rhythms (Lefcourt & Adams 1998) and infrared thermography (IRT) which has been used successfully to measure the surface temperature of cattle in a variety of situations (Coppola *et al* 2002; Berry *et al* 2003; Zahner *et al* 2004). A range of other physiological and behavioural measures that have been implicated in responses to stress, cold exposure, negative energy balance and muscle damage were taken in the present study including plasma cortisol (Ekpe & Christopherson 2000), thyroxine (T4) (Christopherson *et al* 1979), non-esterified fatty acids (NEFA) (Young 1975) β-hydroxybutyrate (BOH) (Clark *et al* 2005), creatine kinase (CK) (Lefebvre *et al* 1996) differential white blood cell counts (Anderson *et al* 1999) and cytokine expression in lymphocytes in response to a vaccination challenge. Behaviour of cattle is influenced by cold temperatures (Gonyou *et al* 1979; Dunn *et al* 1988; Redbo *et al* 2001) and in this study we measured the proportion of time spent standing, lying and eating.

The experimental approach was to compare indicators of cold stress in dairy cows exposed to a week of windy and wet weather with little or no shelter during winter, with those of cows that were housed indoors in a barn.

Materials and methods

The experiment was carried out on a commercial farm near Taupo, New Zealand. All procedures involving the animals used in this study were approved by the AgResearch Animal Ethics Commitee in accordance with the New Zealand Animal Welfare Act 1999. Twenty non-pregnant, non-lactating Holstein Friesian cows were randomly divided into two groups of ten. After a pre-treatment week indoors, each group was exposed to a week of wind and rain (WR) or indoor (I) conditions followed by a post-treatment week indoors in a cross-over design, giving a total of five weeks (Table 1). Wind and rain were produced outdoors using two large fans which were turned on when the natural wind speed fell below 10 kph and overhead sprinklers that were turned on for approximately 15 min h^{-1}. Housed conditions were inside a large roofed barn, with partly open ends and sides for ventilation. The outdoor pen was circular with a diameter of 11.3 m and the indoor pen measured 8 × 12.5 m (length × breadth). During the pre-treatment week, cows were acclimatised to the sampling procedure and exposed to the WR treatment for short periods during the day. Both WR and I treatments had continuous dim (< 15 lux) green light overhead to facilitate behavioural observations during darkness.

Pasture silage and water were available *ad libitum*. Food intake was measured for each group on a daily basis by weighing the food offered, subtracting the weight of food remaining on the subsequent day and correcting for the dry matter content. Air temperature and humidity (Vaisala, Helsinki, Finland), wind speed (Maximum, New Bedford, MA, USA) and direction (NRG, Hinesburg, VT, USA), rainfall (Pronamic, Silkeborg, Denmark) and black globe temperature (Campbell Scientific Inc, Logan, Utah, USA) were recorded every ten minutes with a datalogger (Campbell Scientific Inc, Logan, Utah, USA) on stations in the centre of each pen. As an estimate of the additional chilling effects of wind, a wind-chill value was calculated based on the equation of Environment Canada, with a minor modification so that wind chill equalled the air temperature at wind speeds of less than or equal to 1 km h^{-1}:

$$W = 13.12 + 0.6215 \times T_{air} - 11.37 \times V^{0.16} + 0.3965 \times T_{air} \times MAX(1,V)^{0.16}$$

Where, W is the wind chill, T_{air} is the air temp (˚C) and V is wind speed (kph).

Time spent standing, lying and eating were recorded by trained observers for 16 h per day with instantaneous scan sampling every ten minutes from 1600 to 0800h.

Each day at 0800h, blood, faeces and infrared temperatures were sampled during treatment weeks 2 and 4, and three times per week during weeks 1, 3, and 5. Bodyweights were recorded during this sampling period twice weekly (Tru-Test®). During treatment weeks, WR cows were brought into the barn for sampling. Collection of infrared images began ten minutes after entry to the barn to reduce the effects of direct solar radiation. Infrared images of the orbital and dorsal regions were collected with a FLIR Inframetrics broadband 760 camera, (Inframetrics Corp, North Billercia, MA, USA) calibrated to ambient temperature and humidity conditions, with temperature ranges set on 20˚C for the orbital and 50˚C for the dorsal scans. The upper and lower temperature limits during recording were selected to include the entire range of temperatures of the objects. The resolution was 0.1˚C and accuracy ± 2˚C for these temperature ranges. Infrared images were collected continuously for 5 s onto digital video (Sony DCR-TRV39). Three random images from each video scan were selected and converted to a 256 greyscale image in which each shade of grey corresponded to a specific temperature. The images were then analysed using ImageJ software (version 1.32, National Institute of Health Services Branch, Bethseda, MD, USA). For the orbital region the maximum temperature of the entire eye area was recorded. For the dorsal region a fixed square area (box), was placed so that the spine ran down the centre of the box lengthways, and the hips created a line through the centre of the square widthways.

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Table 2 Bovine specific primers used in this study.

Gene	Primer sequence	Length	GenBank reference
β2-microglobulin (B2M)	Forward cca tcc agc gtc ctc caa a Reverse ttc tcc cca ttc ttc agc aaa	136 bp	X69084
Heat shock protein 90 (SP90)	Forward tga cga gga tga ccc cac tg Reverse tgg agg gaa tgg aga cag agc	159 bp	CB223905
Tumour necrosis factor (TNF)	Forward taa caa gcc ggt agc cca cg Reverse gca agg gct ctt gat ggc aga	277 bp	NM173966
Interleukin 6 (IL-6)	Forward tcc aga acg agt atg agg Reverse cat ccg aat agc tct cag	236 bp	NM173923
Interferon gamma (IFN-γ)	Forward ata acc agg tca ttc aaa gg Reverse att ctg act tct ctt ccg ct	218 bp	M29867

Internal body temperature was recorded every ten minutes using a Vemco TX minilog data logger (Vemco Ltd, Shad Bay, Nova Scotia, Canada) attached to a modified CIDR™ (Inter-Ag Pty Ltd, Hamilton, NZ) and placed into the vaginal cavity of all cows on the day prior to treatment and removed on the last day of treatment.

Faecal samples were collected by digital rectal palpation, placed immediately on ice then frozen until assayed for cortisol as described previously (Morrow *et al* 2002). The minimum detectable level was 3.9 $ngml^{-1}$. The intra-assay CVs for plasma pools measuring 115 and 850 $ngml^{-1}$ were 28.6 and 8.1% respectively and inter-assay CVs (n = 5) for the same pools were 26.6 and 14.9%.

Blood samples were collected from the caudal vein by venepuncture into a 10 ml heparin and a 5 ml EDTA vacutainer using 20 g × 3.2 cm needles. Smears were made from whole blood to count white blood cells, eosinophils, monocytes, lymphocytes, segmented neutrophils and basophils. Heparinised blood was centrifuged at 2,000 rpm for 15 min, plasma aliquoted off and stored at –18˚C until assayed.

Cortisol was measured using a double-antibody radioimmunoassay as described previously (Fisher *et al* 2001). The minimum detectable level was 0.37 $ngml^{-1}$. The intra-assay CVs for plasma pools measuring 5.0, 44.3 and 83.0 $ngml^{-1}$ were 13.5, 17.7 and 17.5%, respectively and inter-assay CVs (n = 2) for the same pools were 5.9, 0 and 0%.

Total thyroxine (T4) was measured by a solid-phase, chemiluminescent assay using an Immulite kit (Biomediq-DPC) with interassay CVs of 7, 7 and 5% at 20, 80 and 150 nmol l^{-1}. Commercial colorimetric kits were used to measure β-hydroxybutyrate (BOH, Randox Laboratories, Crumlin, UK), creatine kinase (CK, Roche diagnostics, Indianapolis, USA) and non-esterified fatty acid (NEFA, Wako Chemicals, Richmond, USA) using a spectrophotometric auto-analyzer (Hitachi 717, Hitachi Ltd, Tokyo, Japan). Inter-assay CVs were less than 5% at 2 and 3 mmol l^{-1} for BOH, at 250 IU l^{-1} for CK and at 0.7 mmol l^{-1} for NEFA.

All cows were challenged with 2 ml Leptoshield Vaccine (CSL Animal Health, Australia) subcutaneously after 3 days of cold exposure to test immune responses. The cows had all been exposed to the vaccine within the previous two months. Blood cells were counted using an ADVIA cell counter (Bayer, New York, USA) and differential cells determined under microscopy. Gene expression of bovine specific primers designed for the heat shock protein 90 (HSP90), tumour necrosis factor (TNF), interleukin 6 (IL-6), interferon gamma (IFN-γ) and β2-microglobulin (B2M) genes (Table 2) were measured as described previously (Ireland *et al* 2004). Relative gene expression was calculated by comparing the expression levels just prior to vaccination to the levels on days 1–8 after vaccination, of the target genes (HSP90, TNF, IL-6 and IFN-γ normalised to the expression of house-keeping gene (B2M) and adjusted for efficiencies of amplification (Pfaffl 2001).

Statistical analysis

Treatment effects were examined using ANOVA. Week one (pre-treatment) was analysed to check for initial differences between the treatment groups. The difference between the pre-treatment week and the treatment week was calculated and analysed. To test for post-treatment effects the difference between the treatment week and the post-treatment week was calculated and analysed. ANOVA for a cross-over design was used with cow, week and cow.week as block effects and WR or I as the treatment effect. Each week was also analysed separately with ANOVA using the pre-treatment week as a covariate.

Internal body temperature was modelled using Fourier analysis and the effect of body condition on the temperature measures was assessed by fitting a REML model with random effects of animal and period and fixed effects for treatment and condition score.

Results

The average weather conditions during the treatment periods are summarised in Table 3. The first week of WR treatment was colder than the second treatment week with average air and black globe temperatures 2˚C lower and wind chill 1.5˚C lower than the second week.

The average bodyweight of the cows increased slightly during the experiment from 511 ± 10.0 to 538 ± 11.4 kg. Food intake averaged 12.9 ± 0.33 kg DM cow^{-1} day^{-1}.

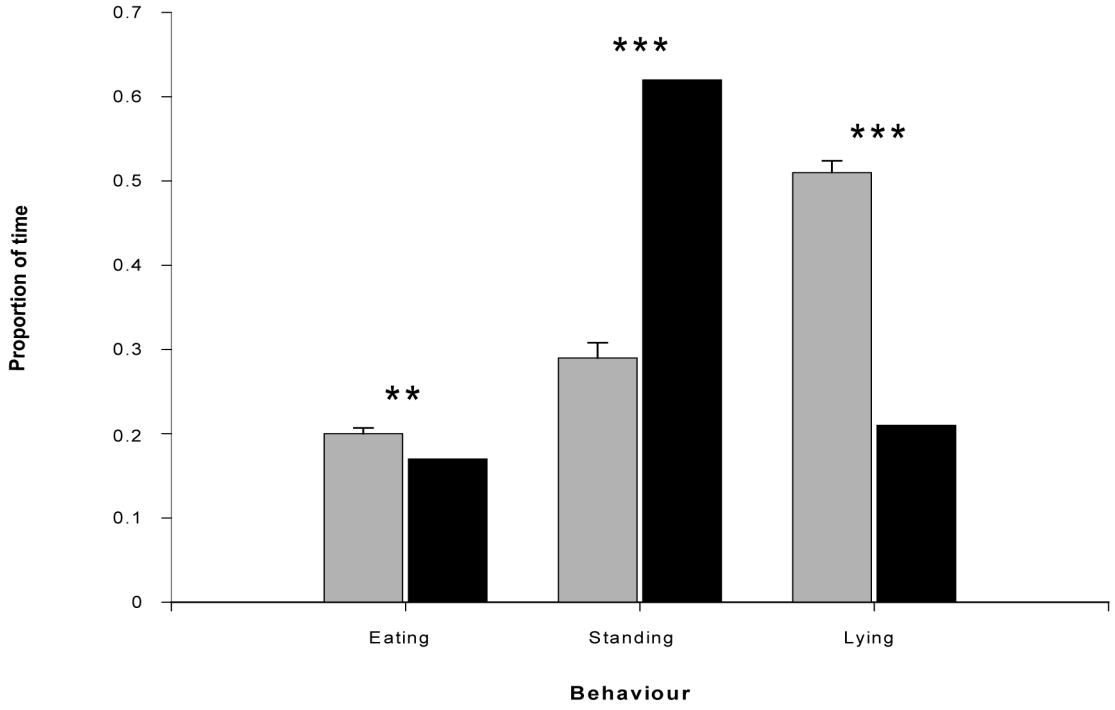
Cows spent a greater proportion of time standing (0.62 vs 0.29, SED 1.8, $P < 0.001$), and less time lying down (0.21 vs 0.51, SED 1.4, $P < 0.001$) in WR than I (Figure 1). The proportion of time spent eating was also less in WR than

https://doi.org/10.1017/S0962728600031948 Published online by Cambridge University Press

Table 3 Weather conditions for cows exposed to wind and rain or housed indoors during the two treatment weeks.

Weather factor	Wind and rain	Indoor	Wind and rain	Indoor	Wind and rain	Indoor
	Mean		Minimum		Maximum	
Air temp (°C)	3.4	4.7	–3.7	–1.3	10.3	13.1
Humidity (%)	83.2	79.4	43.5	44.4	96.5	92.3
Wind speed (kph)	7.1	n/a	0	n/a	23.6	n/a
Rainfall (mm h^{-1})	3.0	n/a	0	n/a	132	n/a
Wind chill (°C)	–0.3	n/a	–7.7	n/a	6.8	n/a

Figure 1



Proportion of time spent eating, standing or lying between 1600 and 0800h for dairy cows exposed to wind and rain (black shading) or housed indoors (grey shading). ** $P < 0.01$, *** $P < 0.001$.

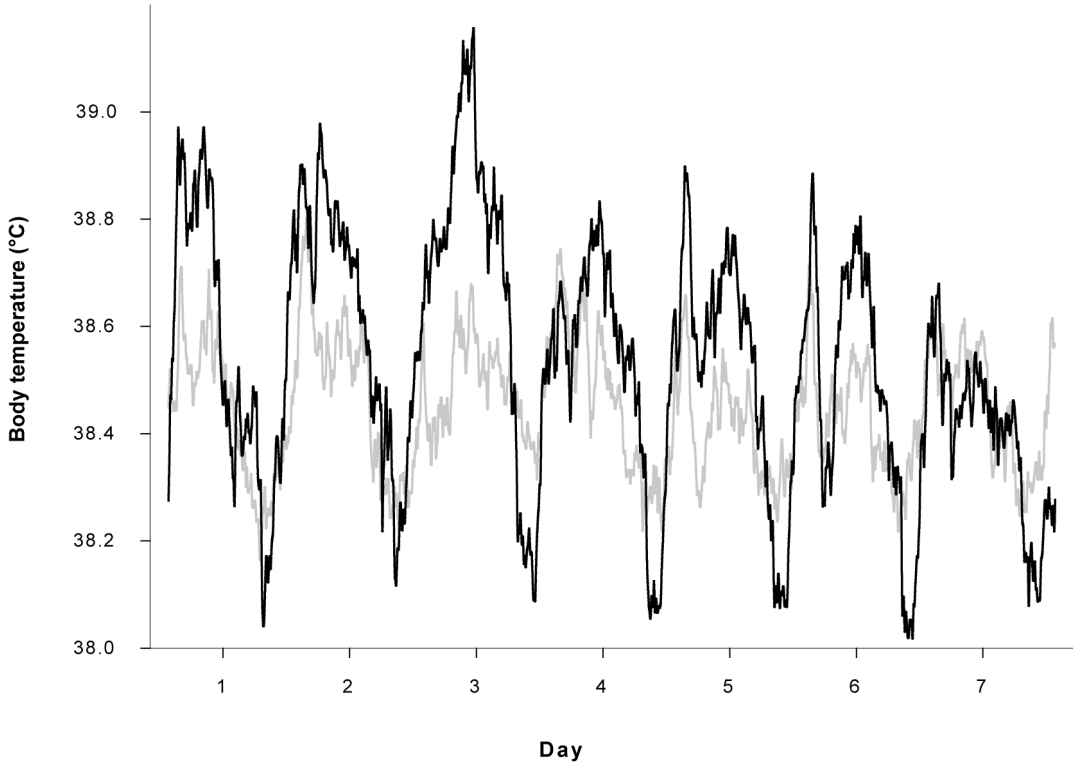
I (0.17 vs 0.20, SED 0.7, P < 0.01), however this was mainly due to a marked difference between midnight and 0800h ($P < 0.0001$) as opposed to before midnight when there was no difference between treatments. Infrared dorsal temperatures (9.8 vs 14.8˚C, SED 0.30, $P < 0.001$) and maximum infrared orbital temperatures (29.5 vs 31.5˚C, SED 0.13, $P < 0.001$) were lower in WR than I.

Internal body temperature underwent a well-defined diurnal pattern (Figure 2) which had a greater amplitude in WR than I, resulting from a lower minimum and higher maximum ($P < 0.001$; Table 4). There was a characteristic double peak in temperature, one occurring in the afternoon at 1547h and a second in the evening at 2145h. The time of the morning minimum occurred 40 min later in WR than I (0942 vs 0902h, SED 0:18, $P < 0.05$). The overall mean body temperature was 0.07°C higher in WR than I ($P < 0.01$).

There was a greater increase in plasma cortisol levels in WR than in I (3.23 vs 0.05 $ngml^{-1}$ (SED 0.894, $P < 0.01$). This effect was more pronounced during the first period of WR (Group 1, $P < 0.05$) than the second (not significant). Faecal cortisol increased by 2.07 vs 0.62 $ngml^{-1}$ (SED 0.551, $P < 0.05$) during WR and I, respectively. Total T4 was higher in WR than I (9.0 vs 3.1 nmol l^{-1}, SED 2.03, $P < 0.01$) and this effect was also greater during the first period of WR ($P < 0.05$) than the second (not significant). The effects on NEFA and BOH were delayed and inconsistent. NEFA concentration was higher in the week following WR than I (0.11 vs 0.09, SED 0.006, $P < 0.001$). While this effect was pronounced after the first period of WR, during the second period there was a small ($P < 0.05$) but opposing effect. BOH underwent a larger drop in WR than I after the first period of WR ($P < 0.05$) and a larger increase in WR than I after the second period ($P < 0.05$).

https://doi.org/10.1017/S0962728600031948 Published online by Cambridge University Press

Figure 2



Mean daily internal body temperature for dairy cows exposed to wind and rain (dark line) or housed indoors (grey line) during week two. Day numbers are positioned at midnight.

Table 4 Mean internal body temperatures for cows exposed to wind and rain or housed indoors during the two treatment weeks.

	Wind and rain	Indoors	SED	Level of significance
Mean (°C)	38.51	38.44	0.027	< 0.01
Minimum (°C)	38.08	38.24	0.030	< 0.001
Time of minimum	0942h	0902h	0018h	< 0.05
Afternoon maximum (°C)	38.74	38.59	0.029	< 0.001
Time of afternoon maximum	1551h	1543h	0011h	0.463
Evening maximum (°C)	38.72	38.63	0.034	< 0.05
Time of evening maximum	2142h	2148h	0026h	0.827

Two peaks in temperature were seen; one close to 1600h and one close to 2200h. SED: standard error of the difference of the means.

Total white blood cell numbers were lower in WR than I (6.98 vs 7.46 × 109 l^{-1}, SED 0.19, $P < 0.05$) and this was mostly due to a reduction in lymphocytes and basophils in WR. Lymphocytes declined by –0.60 and –0.08 × 10^9 l^{-1} (SED 0.22, $P < 0.05$ for WR and I respectively) and numbers were lower in WR than I (3.22 vs 3.53 × 10^9 l^{-1}, SED 0.84, $P < 0.05$). Basophil numbers decreased by –0.025 vs 0.019 × 10^9 l^{-1} (SED 0.015, $P < 0.01$) in WR and I respectively. This latter effect was more pronounced in the first period of WR ($P < 0.05$) than the second (not significant).

No treatment differences were found for CK or for TNF, HSP90, IL-6 or IFN-γ expression in response to vaccination.

Discussion

The combination of wind chill and wetness resulted in a number of behavioural and physiological responses in New Zealand dairy cows. Many of the responses were more pronounced during or following the first (colder) treatment period, a further indication that these changes were due to cold and wet conditions.

https://doi.org/10.1017/S0962728600031948 Published online by Cambridge University Press

IRT measurements of skin and eye temperatures demonstrated that the cows exposed to wind and rain were colder. Knížková *et al* (2002) found that vascular responses of cows to changes in microclimate such as air temperature and air velocity, resulted in changes in the surface temperature of cows, measured using IRT. A close relationship between the environment and surface temperature of cattle as measured by IRT has also been reported by Zahner *et al* (2004). Recent studies indicate that IRT is capable of measuring stress, as an elevated temperature of the capillary bed in the corner of the eye correlated with the cortisol response to ACTH injection in horses (Cook *et al* 2001) and this response occurred during velvet removal in deer (Cook *et al* 2005). We found no evidence of elevated orbital temperature consistent with a stress response during wind and rain exposure in the present study, despite there being other physiological signs of stress. Rather, infrared orbital temperatures appeared related to environmental temperature. Recent results have indicated that hypothalamo-pituitary adrenal axis (HPA) activation does not drive the changes in orbital temperature alone and that psychological aspects of stress may be required for this response (Stewart *et al* 2005). Cold and wet conditions may, therefore, be more of a physiological stress than a psychological one. We cannot discount the possibility that environmental conditions may have overwhelmed an increase in orbital temperature or that our daily sampling regime was too infrequent to detect one.

Internal body temperature monitoring revealed that cows exposed to wind and rain were both hotter and colder than cows indoors due to an increase in the amplitude of the circadian body temperature rhythm. It may be that the lower minimum temperature is simply a result of the greater environmental cooling effect during the period that body temperature naturally falls and the higher maximum is a more vigorous response to this lower temperature during the period that body temperature naturally rises. The net effect was a higher mean temperature during exposure to cold and wet conditions. An elevated mean core body temperature was also found in beef cattle exposed to short bouts of cold (Bergen *et al* 2001). It was later shown by these authors that the body temperature response to short bouts (10 h) of cold is transient, lasting around 5 h (Kennedy *et al* 2005). In the present study there were two peaks in body temperature, one at around 1600h and the other near 2200h. This pattern was almost certainly produced by a thermic response to feeding during the early afternoon (Orskov & MacLeod 1990), superimposed on a normal circadian temperature rhythm (Refinetti & Menaker 1992). Body temperature in cattle appears to have a pattern that is influenced by environmental and physiological conditions and there is clear evidence of a circadian rhythm, although the timing and size of the peaks and troughs varies (Bitman *et al* 1984; Lefcourt & Adams 1996; Lefcourt & Adams 1998). The increased amplitude of the evening peak in cold and wet conditions may have been a hyperthermic response. Bergen *et al* (2001) found the size of the increase in body temperature in cattle was related directly to the severity of the overnight environment. The body temperature measured in our study had a similar mean but lower amplitude (0.64 and 0.39°C for WR and I, respectively) compared to those previously reported for cattle (Piccione *et al* 2003). The later time of the minimum body temperature under cold and wet conditions represents a phase shift in the normal rhythm. Disturbances to biological rhythms are a consistent response to stress and as such represent an imbalance that may predispose animals to disease (Meerlo *et al* 2002). Evidence of disrupted rhythms in response to cold stress may therefore indicate potential for reduced welfare.

The increased cortisol levels found in the present study indicate that a classic stress response involving activation of the HPA occurred in response to cold and wet conditions. Previous studies have found little effect of cold on cortisol in cattle, but marked effects in sheep (Ellis *et al* 1985; Ekpe & Christopherson 2000). The cold and wet conditions may have resulted in the elevated cortisol indirectly, via the reduction in lying time (Ladewig & Smidt 1989; Munksgaard *et al* 1999; Fisher *et al* 2002), however it is likely that a combination of cold, wind and wet conditions may be more stressful to cattle than simply cold alone. The reduction in white blood cells especially lymphocytes and basophils indicate that it may be the latter possibility that is most likely. Reduced lymphocytes occur in cattle as a result of stress caused by transport (Murata *et al* 1987; Tarrant *et al* 1992) or social isolation (Hopster *et al* 1998). Thus, cold and wet conditions which result in decreased lying times caused a potential decrease in immunity which is a more severe effect than that found with reduced lying time alone (Fisher *et al* 1999).

The main behavioural responses of cows to cold and wet conditions were reductions in both lying and eating time. Cattle exposed to cold appear to either increase or decrease lying time (Malechek & Smith 1976; Gonyou *et al* 1979; Redbo *et al* 2001). It has been suggested that the bedding surface may be responsible for this difference, with a wet surface inhibiting lying down (Gonyou *et al* 1979). This is further evidence that wet conditions may exacerbate stress by reducing the time that the animal lays down and rests (Fisher *et al* 2002). A lying proportion of 0.2 under cold and wet conditions, which equates to less than 5 h per day, is well below the normal levels for dairy cows (Jensen *et al* 2005). While absolute daily times for behaviours must be treated with caution as our observations only covered 16 h, the calculated lying time for cows housed indoors in the present study (at around 12 h) is similar to that reported for cattle indoors (Krohn & Munksgaard 1993). The reduced eating time in the present study is consistent with some other studies which found reduced eating or grazing (Malechek & Smith 1976; Redbo *et al* 2001) although once again this appears to be a variable response as little or no effect of temperature on grazing time has been reported in other studies (Dunn *et al* 1988; Beverlin *et al* 1989; Prescott *et al* 1994). There is evidence that cold increases eating rate in sheep (Kennedy 1985), however, intake and daily gain in cattle are generally related to feeding duration

https://doi.org/10.1017/S0962728600031948 Published online by Cambridge University Press

(Schwartzkopf *et al* 2002). It is, therefore, likely that the decreased feeding time in response to cold and wet conditions corresponded to reduced feed intake which could further impact on the animal's thermoregulatory ability.

Thyroid function is elevated by cold exposure in both sheep and cattle (Kennedy *et al* 1977; Christopherson *et al* 1979; Horton 1981; Scott & Christopherson 1993) which was borne out in the present study. Similarly an increased secretion of NEFAs in response to cold was seen which is consistent with previous studies (Fujita *et al* 1982; Broucek *et al* 1987). Both of these responses indicate that metabolic adaptations to cold exposure occurred.

In conclusion, short-term exposure to cold and wet conditions resulted in a number of changes in dairy cows that indicate activation of the stress axis, physiological and behavioural adaptive responses to cold and a reduction in welfare. The measures used in the present study could form part of a toolkit to assess welfare of dairy cattle on farms. The lack of dry lying space appeared to exacerbate the stressful effects of cold and wet weather by reducing lying time. Provision of shelter and dry surfaces for lying appears to play an important role in mitigating the impact of cold and wet weather on pastoral dairy farms.

Acknowledgements

The authors would like to thank the following for their assistance with this study; LandCorp Ltd, for the use of their farm, facilities and staff, AgResearch staff for technical assistance, Dr R Cursons, Waikato University for cytokine analysis, the Foundation for Research, Science and Technology and Dairy InSight for funding.

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https://doi.org/10.1017/S0962728600031948 Published online by Cambridge University Press