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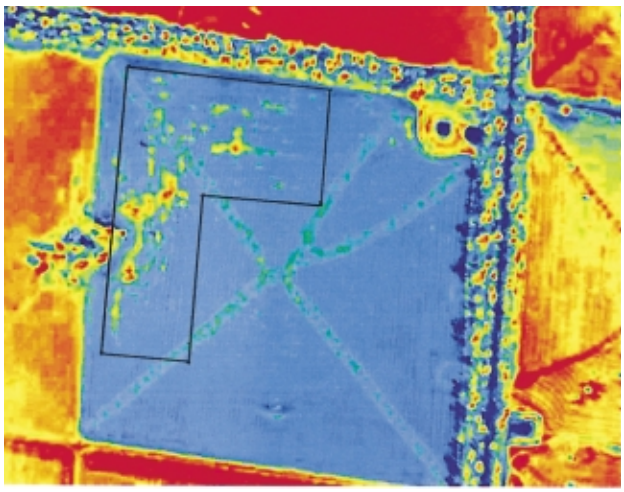

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Associations between immune system, growth and carcass variables in cattle

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Abstract. A range of haematological and immunological variables was measured in calves to assess relationships between these factors and effects of maternal and pre-weaning supplementation on meat quality. The ability of immunological and haematological measurements to predict both weight loss during transport and meat quality was also examined. Calves from supplemented cows had higher counts of lymphocytes bearing the γδ T cell receptor [workshop cluster (WC)1+ lymphocytes]. When cultured *in vitro*, lymphocytes from calves supplemented directly produced higher levels of the cytokine interferon (IFN)γ. These changes may reflect a greater resilience to the effects of stress associated with routine handling for weighing and blood sampling, or earlier maturation of the immune system in supplemented calves. Two calves from unsupplemented treatments died of calf diphtheria before weaning. During feedlot finishing, the prevalence of disease was low and did not differ between groups. The prevalence of cluster of differentiation (CD)4+ lymphocytes in blood was correlated with growth rates from weaning to slaughter and during feedlot finishing. Lymphocyte counts and the ratio of neutrophils to lymphocytes in blood collected either before or after transport was correlated with weight change during transport. These variables were not correlated with meat quality, however the numbers of lymphocytes after transport in the subset bearing the CD8 marker was correlated with the meat quality traits, tenderness, juiciness, flavour, overall likability, meat quality (MQ) 4 score and Meat Standards Australia (MSA) grade ratings. The results demonstrate that lymphocyte subpopulations are associated with growth rate, weight loss during transport, and meat quality. Their utility in predicting performance, stress tolerance, and meat quality therefore, warrants further investigation.

Introduction

Cattle respond to stressors through changes in their physiological status and in their behavioural repertoire. Prolonged exposure of cattle to a stressor may lead to pathologies such as metabolic and infectious diseases, behavioural stereotypes and reproductive malfunctions (Moberg 1985). Furthermore, meat quality may be reduced when exposure to stressors immediately precedes slaughter (Tarrant 1988). Thus, an ability to predict the response of individual animals to future stressors may have utility for the beef industry, especially if associated with production performance or meat quality.

In addition to stressors and immune stimuli, the genetic makeup (Bishop *et al*. 1992) and nutritional status (van Houtert and Sykes 1996) of an animal can influence haematological and immunological variables. When an animal perceives a stressor, neuroendocrinological events that modulate metabolic, behavioural and immunological systems within the body are stimulated (Moberg 1985). Monitoring changes in these systems provides an indication of the impact of the stressor. Limitations to measuring the impact of stressors can arise due to lability of the changes, difficulty in their quantification, or susceptibility to extraneous effects. As an adjunct to more conventional measures such as behaviour and plasma cortisol concentrations, the utility of examining changes in immune function for monitoring the impact of stressors has recently attracted attention in a number of laboratories (Burton *et al.* 1995; Amadori *et al.* 1997; Anderson *et al.* 1999).

Calves bred on the Northern Rivers of New South Wales often have low weaning weights and fail to exhibit sufficient compensation during subsequent growth to reach slaughter weight at the same time as their heavier-weaned siblings (Hearnshaw *et al.* 1995). The current study was undertaken in conjunction with an examination of the effect of pre-weaning supplementation on carcass yield and meat quality (D. W. Hennessy and S. G. Morris unpublished data). Calves reported in this paper received pre-weaning supplementation to examine the capacity of strategic nutritional treatments to improve longer-term growth performance and meat quality. At the same time, blood samples were collected to examine the hypothesis that haematological and immunological variables may be used to predict growth rates, response to transport and meat quality.

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Materials and methods

Animals

Animals in this experiment received treatments as described in detail by D. W. Hennessy and S. G. Morris (unpublished data). Cattle were run until feedlot entry at NSW Agriculture's Agricultural Research and Advisory Station, Grafton, New South Wales. Briefly, the study was performed on 124 mixed sex calves and their dams (100 Angus × Hereford calves born to Hereford cows and 24 Hereford calves). There were 3 calf treatments and 2 dam treatments in a replicated, factorial design, with 4 replicates of pre-weaning treatments (30 calves per replicate with 5 or 6 calves per cell). Calf treatments were nil or creep feed of a proprietary high crude protein (22% as fed) supplement (Graz-R 30 calf pellets Ridley AgriProducts Pty Ltd, Wacol, Qld) from 75 or 150 days of age with an average daily intake of 2 kg per calf. Dam treatments were nil or twice per week supplementation with 4.2 kg cottonseed meal for 145 days from 50 to 60 days before anticipated calving date. As a requirement of care and ethics guidelines there was close monitoring of animal health and efforts were taken to minimise stress on cows and calves when mustered for weighing and blood sampling. Following weaning at 210 days, calves remained at Grafton and rotationally grazed paddocks containing a high quality ryegrass pasture for 7 months. They were then transported 320 km to the 'Tullimba' Cattle Research Facility, near Armidale, NSW, for finishing on grain-based diets. Experienced pen riders examined cattle each morning for signs of inappetance and morbidity. When cattle were 'finished', they were removed from the facility, either after 84 days (n = 77) or 91 days (n = 36) and transported 380 km by 'TruckCare'-accredited, double-deck transports to the Northern Co-operative Meatworks, Casino, for slaughter. The 1-week interval between slaughters resulted from a limit on carcass numbers imposed by the processer in its roster at the commercial abattoir used in this study. Carcasses were electrically stimulated, graded on Meat Standards Australia (MSA) criteria and striploin portions from half the carcasses were removed for assessment of tenderness, juiciness, flavour and likability. The weighted sum of these scores is termed the MQ4 index, according to Meat Standards Australia guidelines (Polkinghorne *et al*. 1999). Meat was graded according to the MQ4 index as MSA Tenderness, MSA Premium Tenderness and MSA Supreme Tenderness.

Objective measurements of meat quality

Objective measurements on meat were made as follows. Trimmed blocks were weighed and placed in plastic bags and cooked in a 70°C waterbath for 60 min. Samples were cooled under cold running water for 30 min and stored overnight at 1°C before weighing and cutting 6 cross-sectional slices for Warner-Brazler shear, and 2 wedges for compression tests, using a Lloyd measuring instrument with modified Warner-Bratzler and compression attachments (Bouton and Harris 1972). For both peak force and compression measurements 6 readings were recorded and averaged for each sample. Cooking loss was expressed as the loss in weight during cooking as a proportion of pre-cooked weight.

Weighing procedures and haematological and immunological assays

Of the 120 calves in the pre-weaning phase of the experiment, 60 representing 2 of the 4 replicates were serially bled at 75, 150 and 210 days of age and 109 were bled at 414 and 421 days of age. All calves were mustered and weighed at a mean age of 112 days when the male calves were castrated by surgical excision. Day 210 bleeds coincided with the day calves were removed from their dams for weaning. The latter 2 bleeds occurred before and immediately after transport from Grafton to 'Tullimba'. Animal weights were measured directly off feed with the exception of the post transport weight on day 421 that was taken when cattle were off-loaded from trucks. Weight loss following transport was calculated as day 421 weight – day 414 weight. Blood was collected in vacutainers containing disodium ethylene diamine tetraacetic acid (EDTA) as an anticoagulant for haematology and a range of immune function assays. Total leucocyte counts and red cell variables were measured on a Coulter counter S880 calibrated for cattle blood. Differential leucocyte counts were performed by flow cytometry on a FACS Vantage flow cytometer (Becton Dickinson, San Jose, CA, USA) on the basis of CD14 staining and leucocyte side scatter as described by Anderson *et al*. (1999). Lymphocyte proliferation to the mitogens concanavalin A and phytohaemagglutinin, production of the cytokine IFNγ, monoclonal antibodies and procedures used for leucocyte staining with the cell surface markers CD4, CD8, WC1, IL-2Rα and L-selectin were performed as described by Anderson *et al*. (1999).

Statistical analyses

The effect of dam and calf treatment groups was analysed in the statistical package Systat (Systat Inc, Evaston IL, USA) with a general linear model for repeated measures fitting cow treatment, calf treatment, sex, and cow treatment × calf treatment interaction. Non-significant effects were retained in the model. Spearman rank correlations between variables were assessed in the statistical package Sigmastat (SPSS Inc, Chicago IL, USA). $P<0.05$ was considered significant and $0.05<P<0.10$ was considered to indicate a trend.

Results

Effect of supplementation on growth, immunological and haematological measures

Calves suckled by supplemented cows were heavier at weaning (201 *v.* 170 ± 4.4 kg) and at slaughter (444 *v.* 425 ± 7.3 kg; D. W. Hennessy and S. G. Morris, unpublished data). At weaning, the following erythrocyte variables were higher in calves suckled by supplemented cows: red cell counts (supplemented dams, 9.0 ± 0.20 $\times 10^9$/mL, unsupplemented dams 8.0 ± 0.18 $\times 10^9$/mL, $P<0.01$), haematocrit (supplemented dams, 29.5 ± 0.66%, unsupplemented dams 27.0 ± 0.60%, $P<0.05$) and haemoglobin concentrations (supplemented dams 11.5 ± 0.23 g/dL, unsupplemented dams 10.4 ± 0.23 g/dL, $P<0.01$). The percentage of WC1+ lymphocytes was higher at 150 days ($P<0.01$) and tended to be higher at weaning ($P<0.1$) in calves suckled by supplemented cows, whereas the percentage of CD4+ lymphocytes was lower in calves suckled by supplemented cows at 150 days ($P<0.05$) (Fig. 1). Lymphocyte proliferation did not differ but production of the cytokine IFNγ was higher at weaning in both calf treatments receiving supplementation (Fig. 1). These variables did not differ between treatment groups at 414 days.

Correlations of blood variables with growth rates

From blood collected at 75 and 210 days of age, there was a tendency for the percentage of CD4+ lymphocytes to be negatively correlated with growth rate from weaning to slaughter (Table 1). From blood collected at days 75 and 150 of age there was a tendency for the percentage of CD4+ lymphocytes to be negatively correlated with growth rate from feedlot entry to slaughter (Table 1). Other blood variables were not significantly correlated with growth rates at more than one blood sampling time.

Effect of transport on weight and blood variables

Cattle were significantly lighter (mean ± s.e.) following transport (324.9 ± 3.8 kg before and 309.2 ± 3.7 kg after transport, n = 103, P = 0.002). After transport, there were significant increases in red cell count, neutrophil percentage, monocyte percentage and neutrophil : lymphocyte ratio, and a significant decrease in the lymphocyte percentage (Table 2). Other haematological and immunological measurements did not differ.

Correlations of blood variables with transport-induced weight loss

At 75 days of age, red cell count, haemoglobin concentration and haematocrit were negatively correlated with transport-induced weight loss (Table 1). At 150 days of age CD4+ lymphocyte percentage was correlated with weight loss and at 210 days of age haemoglobin concentration and haematocrit were correlated with weight loss (Table 1). Immediately before transport, the percentage of neutrophils positive for the marker L-selectin, lymphocyte percentage and neutrophil : lymphocyte ratios were correlated with subsequent weight loss (Table 1).

From blood collected following transport, lymphocyte percentage, monocyte percentage, neutrophil percentage and neutrophil : lymphocyte ratios were correlated with the weight loss (Table 1).

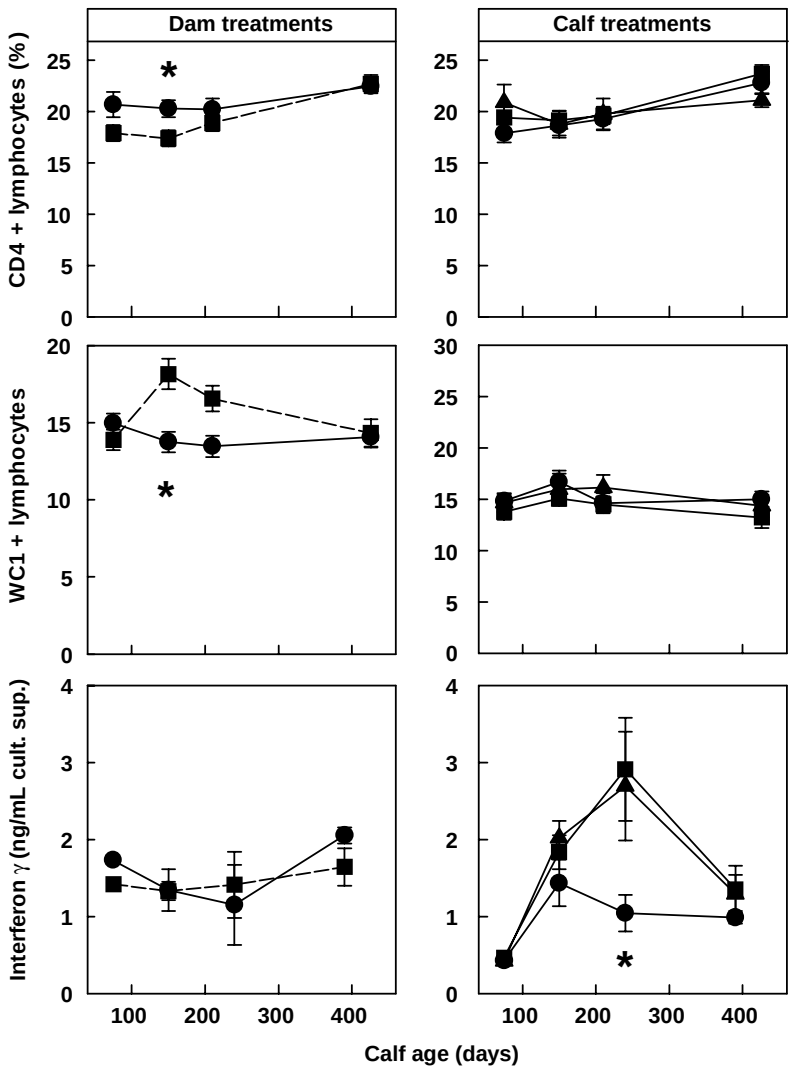

Figure 1. Effect of dam (● nil, ■ supplemented) and calf treatments (● nil, ■ supplemented from day 75, ▲ supplemented from day 150) (60 calves per dam treatment group and 40 calves per calf treatment group; see Materials and methods for details) on prevalence of CD4+ and WC1+ lymphocytes in calf blood and production of the cytokine interferon γ during *in vitro* lymphocyte culture. Asterisk indicates that differences between means are significant at P = 0.05.

Table 1. Spearman rank correlations indicating significance or a trend between blood variables and growth rates, transport-induced weight loss, and meat quality

Variable 1	Variable 2	*r*	*P*
	Correlations with growth rates		
CD4+ lymphocytes at day 75	Growth rate weaning to slaughter	–0.2574	0.068
CD4+ lymphocytes at day 210	Growth rate weaning to slaughter	–0.2715	0.056
CD4+ lymphocytes at day 75	Growth rate feedlot entry to slaughter	–0.2817	0.045
CD4+ lymphocytes at day 150	Growth rate feedlot entry to slaughter	–0.2624	0.060
	Correlations with transport-induced weight loss		
Red cell count at day 75	Weight day 421 – weight day 414	–0.3098	0.038
Haemoglobin concentration at day 75	Weight day 421 – weight day 414	–0.3320	0.026
Haematocrit at day 75	Weight day 421 – weight day 414	–0.3276	0.028
CD4+ lymphocytes at day 150	Weight day 421 – weight day 414	0.3017	0.044
Haemoglobin concentration at day 210	Weight day 421 – weight day 414	–0.3439	0.024
Haematocrit at day 210	Weight day 421 – weight day 414	–0.3366	0.028
L-selectin at day 414	Weight day 421 – weight day 414	0.3170	0.002
Lymphocyte % at day 414	Weight day 421 – weight day 414	–0.3106	0.004
Neutrophil:lymphocyte ratio at day 414	Weight day 421 – weight day 414	0.2193	0.044
Lymphocyte % at day 421	Weight day 421 – weight day 414	0.4400	0.000
Monocyte % at day 421	Weight day 421 – weight day 414	–0.2937	0.009
Neutrophil % at day 421	Weight day 421 – weight day 414	–0.2207	0.049
Neutrophil:lymphocyte ratio at day 421	Weight day 421 – weight day 414	–0.2551	0.023

Correlations of blood variables with meat quality

The 4 meat quality traits, *viz.* tenderness, juiciness, flavour and likability, as well as the MQ4 index and Meat Standards Australia Tenderness gradings, were highly correlated ($0.8300<r<0.9600$, all $P<0.0001$). No haematological or immunological measurements were correlated with these traits at more than 1 of the 3 pre-weaning sampling times. From the blood sample collected following transport, the percentage of CD8+ lymphocytes was correlated with all these meat quality traits ($0.2754<r<0.4328$, $0.064<P<0.003$).

No significant correlations were found between the haematological variables affected by the stress of transport and the objective meat quality traits, pH, compression, adhesion and peak force.

Health during the pasture grow-out phase and in the feedlot

Two calves from unsupplemented groups died of calf diphtheria before weaning. One animal died during sampling of the *M. semitendinosus* at weaning and macroscopic pathology implied white muscle disease and heart failure, but these lesions were not confirmed in a diagnostic laboratory. However, blood samples taken on all calves at weaning indicated low activity of glutathionine peroxidase and consequently all animals were given a selenium supplement (Selpor). Despite drenching of calves at weaning, many calves were apparently affected by internal parasites when placed onto ryegrass pastures. All calves commenced a rigorous drenching program (D. W. Hennessy and S. G. Morris unpublished data), which continued for 8 weeks to reduce scouring and allow growth. One weaner died whilst on ryegrass but an examination *post mortem* could not be undertaken. Two heifers suffering 'ill thrift' were removed from ryegrass pastures and were diagnosed as carriers of *Pestivirus* with incomplete seroconversion despite all heifers having equal exposure to the virus. However, no animals required removal from the feed pen for health reasons during finishing in the feedlot and 113 were slaughtered.

Table 2. Haematological variables (mean ± s.e.) affected by transport of cattle 320 km from Grafton to Armidale ($n = 109$)

Variable	Before transport	After transport	*P*-value
Red cell count (10^9/mL)	8.18 ± 0.08	8.54 ± 0.09	0.001
Lymphocyte (%)	64.90 ± 0.84	56.13 ± 0.99	<0.001
Monocyte (%)	5.89 ± 0.28	8.18 ± 0.26	0.015
Neutrophil (%)	22.91 ± 0.81	30.15 ± 0.88	0.004
Neutrophil:lymphocyte	0.373 ± 0.019	0.567 ± 0.024	<0.001

Discussion

At weaning, there were higher levels of WC1+ lymphocytes and higher production of the cytokine INFγ in calves receiving dam or creep supplementation. WC1+ lymphocytes play an important role in immunological surveillance of mucosal surfaces of the body (McClure *et al.* 1989) and are susceptible to suppression by the effects of stress (Burton *et al.* 1995; Anderson *et al.* 1999). The higher percentage of WC1+ lymphocytes in calves of supplemented cows may represent greater resilience of these animals to stressors intrinsic to the experiment such as handling and

weighing. Weaning is a stressful event for calves (Fell *et al.* 1998), and the higher level of WC1+ lymphocytes, together with a greater capacity to produce the cytokine, IFNγ, may help protect supplemented calves from infection at this time. Whilst care was taken to reduce stress associated with husbandry levels it is noteworthy that the 2 calves that died of calf diphtheria were from the unsupplemented control group. Diphtheria is a disease associated with infections of opportunistic bacteria and rarely occurs in healthy animals, with few deaths ever reported. The effects of supplementation on blood variables were largely transitory as few treatment effects were evident at day 414 and health outcomes did not differ between treatment groups during feedlot finishing.

Nutritional effects on erythrocyte parameters have previously been noted (Jain 1993). The life span of erythrocytes in calves at 3 months of age is 48–63 days while in adults it is about 160 days (Jain 1993). The higher values for red cell counts, haemoglobin concentrations and haematocrit observed at weaning in calves from supplemented dams may reflect an influence of dam nutrition on lifespan or production of erythrocytes.

CD4+ lymphocytes were negatively correlated with growth rate from weaning to slaughter and from feedlot entry to slaughter. A physiological basis for this association is not obvious although it is possible that the percentage of CD4+ lymphocytes is suppressed by endocrine factors associated with growth rates.

Transport is well recognised as a stressor that increases susceptibility of cattle to respiratory disease (Grandin 1997). Weight loss during transport can be greater than that associated with fasting for a similar period of time and is increased by administration of adrenocorticotropic hormone, thus an interaction between stress responses and weight loss during transport can occur (Phillips *et al*. 1991). Short transport from Grafton to Armidale, induced changes in haematological parameters in line with those previously reported in the literature (Kent and Ewbank 1986; Tarrant *et al*. 1992). Lymphocyte and neutrophil percentages in blood are particularly sensitive to the effects of stressors, with low neutrophil : lymphocyte ratio after exposure to a stressor reflecting resilience to the stressor (Tarrant *et al.* 1992). Importantly, high lymphocyte counts before transport were associated with reduced weight loss during transport, suggesting that this population of blood leucocytes may have utility in predicting resilience to stress.

Consistent haematological and immunological predictors of meat quality were not found in blood samples taken before weaning. Blood variables associated with resilience to weight loss during transport were not correlated with meat quality. However, a subpopulation of lymphocytes, the CD8+ lymphocytes that are sensitive to the effects of some stressors (Anderson *et al.* 1999), were correlated with subjective meat quality traits. These findings on a small number of cattle suggest that more detailed studies on the associations between lymphocyte populations, growth rates, stress tolerance, and meat quality are warranted.

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