MEAT & LIVESTOCK AUSTRALIA

final report

Project code: B.FLT.0353

Prepared by: Geme Wijffels, John Gaughan, Dean Revell and Peter Allingham
CSIRO and University of Queensland

Date published: February 2013

PUBLISHED BY
Meat and Livestock Australia Limited
Locked Bag 1961
NORTH SYDNEY NSW 2059

Heat Stress Nutrition: A comprehensive review and R&D program

Meat & Livestock Australia acknowledges the matching funds provided by the Australian Government to support the research and development detailed in this publication.

Abstract

The beef feedlot industry has made much progress in preventing fatalities and morbidity as a consequence of heat stress. However, productivity losses persist during and after periods of high heat load. Dietary interventions are seen as part of a solution to ameliorate heat load in growing beef cattle. This review documents past and current efforts to adjust rations (fibre:fat:protein) and the use of additives to reduce heat load. Despite decades of anecdotal observations, and research using pen trials and the controlled environment of the climate chamber, the findings show neither approach has delivered a consistent outcome. A dietary intervention, successful in one situation, is often not reproducible.

What is evident are the advances that have occurred in understanding the endocrine and physiological responses to high heat load. The involvement and possible damage of the gastro-intestinal tract may be central to invoking a system-wide inflammatory response, which, in turn, exacerbates the heat load and stress response. On this basis, the review proposes a 5-year R&D program to:

- Validate that these events occur in feedlot cattle under high heat load;
- Find simple easy-to-measure correlates of severity of damage;
- Trial dietary additives or adjustments that will prevent or reduce damage.

Executive summary

The cattle feedlot industry is cognisant of society's oversight of the welfare of animals in their care, the economic demand for increased productivity, and the increased climatic variability of Australian beef production regions. Over the last two decades, the industry and MLA, through learning from experience and with investment in R&D, have overcome extreme effects of heat wave events. Nonetheless, there is recognition that high heat loads experienced by cattle in feedlots continues to be detrimental to their welfare and growth.

Despite considerable R&D investment by the MLA and researchers internationally over several decades, limited inroads have been made into the development of successful nutritional strategies to offset or ameliorate these impacts of heat stress. Many of the 'off-the-shelf' feed additives have been trialled and found to have no or equivocal improvement to productivity or cattle comfort. These outcomes highlight the necessity of looking deeper into the altered physiology and metabolism of feedlot cattle over the summer period and during heat waves. It has become apparent that rumen and gut health is most likely at the centre of high heat load morbidity and poor recovery after heat stress. The sometimes promising and then disappointing results of these nutritional intervention studies may have arisen from poor understanding and control of the stress-related inflammatory status of the animal. The complexity of needed studies and often time discouraging outcomes deterred investigation into opportunities for the strategic use of nutritional interventions prior to, and through to recovery from acute or chronic (modest but long duration thermal discomfort) heat events. As a consequence, the biology of the animal recovering from hyperthermia is not understood.

With input from industry representatives, a 5-year R&D program was designed to deliver new nutritional strategies for a forecast heat event (including pre-emptive and post-event interventions) and investigate the need for management during summer high risk period and/or all of summer. To ensure that any recommended intervention is applied to gain the optimal result, the program intends to understand the altered metabolic and inflammatory responses induced by high heat load.

On this basis, we have proposed a 2-stage approach for future R&D: firstly, use of very controlled conditions followed by pen trials to validate altered inflammatory and metabolic status and gut barrier disruption; secondly, applying the knowledge from stage 1, nominate and test the potential use of commercially-feasible feed additives that could ameliorate the changed state in the feedlot animal.

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1 Aims and Scope

Simplistically the remit of this review to answer the question “Is there are biological researchable question to address aspects of heat stress in the Australian beef industry?”

More precisely, the purpose of this review was three-fold:

1. To provide a comprehensive review of current and past knowledge on the nutrition and metabolism of cattle exposed to high heat load,
2. To produce a succinct summary of the current global knowledge base on heat stress nutrition in cattle
3. To present a recommended R&D program, extending 5 years that addresses the identified research gaps and opportunities.

A multidisciplinary team was assembled to ensure a breadth of knowledge domains, and fresh ideas to contribute to the literature review and development of the Recommended R&D program. A comprehensive literature review has been undertaken focussing on the metabolic and physiological responses of beef and dairy cattle (and other ruminants as appropriate) when they are exposed to high heat load and attempts to ameliorate the negative impact of heat stress by nutritional interventions. To ensure inclusion of all relevant nutrition and metabolic studies, the review surveyed the scientific and extension literature from the 1950’s to the present (see References, Appendix 8.1). There is a large literature on the use and success of numerous additives that might be classed as micro-nutrients. To limit the span of this topic, the review has included only those additives reported in credible literature sources and assessed by sound experimental method.

Knowledge of current and unpublished research (including on-farm) was gained by site visits to, and interviews, with international researchers active in the field (i.e. applied feedlot nutrition, heat stress physiology, nutrition and/or metabolism in cattle). The visits were very insightful of future investment and capacity for this area of research. The review also includes an audit of Australian and overseas ruminant nutrition research facilities that enable climate controlled experiments and/or microclimate informed animal experimentation.

Information from Australian and US feedlot nutrition consultants provided on-the-ground knowledge and progress in private sector research efforts. Practical knowledge and experience in feedlot management and nutrition was accessed by visiting a research feedyard attached to a large operating feedyard in the USA and through a workshop with Australian feedlot industry nutritionists, managers and veterinarians. In addition, an appropriately qualified overseas consultant, Dr Terry Mader, was contracted as a formal reviewer of the project outputs.

To remain focussed on nutrition during high heat load for feedlot beef cattle, this review has limited coverage of genotype or phenotype influence on thermotolerance (the latter has been well covered in numerous recent reviews), and very little commentary on effects of heat stress on reproduction. Reference has been made to the well-developed science and extension literature on heat stress in dairy cattle where generally the economic and physiological costs have been better researched. Findings from rodent models and human studies are also included where information in ruminant systems is absent.

The literature review covers much ground and goes into some detail; to enable readers to quickly get to the overview and recommendations, we have placed that section earlier in the document (Section 3 – The way forward).

2 Background

Heat stress in livestock has been acknowledged by the animal industries as a production and welfare issue for several decades particularly where producers are operating in warmer climates (reviewed Bernabucci et al., 2010; Bianca, 1965). A number of drivers are encouraging both producers and researchers to focus their attention on managing heat stress in cattle

- more extreme weather events and increasing climate variability
- Increased numbers of cattle in feedlots
- Increased expectations of growth at feedlots and
- Increased societal oversight of animal welfare (Brown-Brandl et al., 2003).

Sustained hot weather and abrupt heat waves both induce heat stress but require different management interventions due to differences in animal responses to these two circumstances. In either case, unacclimatized animals, feedlot animals close to market weight and high producing dairy cows are particularly vulnerable (Hahn, 1999). Long periods of hot weather are known to cause production losses through reduced weight gain and reproductive inefficiencies, however, major heat wave events such as those experienced in Iowa (1995), Nebraska (1999) and California (2006) have caused the deaths of thousands of cattle.

An internal body temperature only 3-6°C in excess of the normal internal body temperature range is fatal for most mammalian species (Silanikove, 2000). Relative to other mammals, cattle are particularly vulnerable to heat stress due to their higher basal metabolic rate and poor water retention by the gut and renal system (Bernabucci et al., 2010). For Bos taurus the critical ambient temperature (threshold temperature) that provokes an increase in internal body temperature is 25°C (Scharf et al., 2010). In most livestock species, only a 1°C increase in rectal temperature will reduce productivity.

The trend toward increased hot conditions in the cattle production regions is clear. Howden and Turnpenny, (1997) report that for the Gayndah region (South East Queensland), the last 40 years have experienced a 60% increase in days that cause heat stress in taurine cattle. On choosing an intermediate warming scenario of an average temperature increase of 2.76°C by 2100, the authors noted that the number of heat stress days will increase to 139 days per annum, as compared to the 58 heat stress days currently experienced. Of real concern is the prediction that by 2100, this region will face 92 days per annum where physiological coping mechanisms in cattle will fail, exposing a high risk of fatalities. A 13% increase in water requirement is also predicted in this study. When Howden et al. (1999) extended this scenario to the whole Australian continent, heat stress days for cattle increased by 10-20% in most cattle producing regions, with an increase of 30% in far northern regions. These regions can anticipate operating in heat stress conditions for all of the year, regardless of season.

Hahn (1999) concluded that much of the research to date has centred on two questions:

- How much harm, immediate or longer term, is associated with a given situation/environment? The research has dwelled more on describing and measuring immediate harm, rather than longer term effects in various cattle breeds, adapted and non-adapted.
- Is there a need for intervention to reduce the risk and level of harm to animals and enterprises?

Fatalities during heat wave events and the now well understood losses to production would indicate that intervention is needed to reduce harm. There has been research into different management tactics and tools with some up take by producers and producer organisations (e.g. MLA, 2006). Additionally, as the costs of energy increase, returns on cooling the animal or its environment have reduced (Collier et al., 2006a). Many researchers point to genetic selection as a means to equip the industry with heat tolerant breeds (Gaughan et al., 2010a; Howden and Turnpenny, 1997; Mackinnon et al., 1991; Collier et al., 2006a). Selective breeding is a long and imprecise process but needs to be part of the answer. However, tools for detecting economically-competitive heat tolerant phenotypes are limited by a lack of knowledge as to which physiological parameters are most appropriate. Furthermore, the technology by which to measure these parameters on numbers of animals in production environments is still under development or not yet in the pipeline.

2.1 National and international industry experience of heat stress production losses and fatalities

The major and most obvious contribution to productivity loss in cattle from heat stress is decreased feed intake and subsequent slower weight gain, decreased efficiency, and reduced milk yield and milk quality. Less obvious are the decreased reproductive performance and increased metabolic disorders (Wheelock et al., 2010). It is apparent that all stages of bovine reproduction are affected by heat load.

The economic impacts of heat stress on the dairy industries in developed countries are relatively well understood. The industry is dealing with decreased heat tolerance due to intense genetic selection for high milk yield cows (Bernabucci et al., 2010). The modern US dairy cow generates more heat than her 1950's counterpart (Collier and Zimbelman, 2007). Under hot conditions average energy maintenance costs for milking cows are estimated to increase by 25-30% (Fox and Tylutki, 1998). Berman (2005) estimated by increasing daily milk yield from 35 L to 45 L, the threshold temperature in these high-yielding animals has been reduced by 5°C. The hotter US states can anticipate decreased milk yields of 1,200-2,100 kg/cow p.a. St-Pierre et al. (2003) assessed the costs of heat stress to the US dairy industry at 900 million US dollars annually despite investment to improve environmental conditions in dairies. Rhoads et al. (2009a) suggested that these estimates are manifestly too low as the 2006 California heatwave alone was estimated to cost the US dairy industry 1 billion US dollars.

Generally, heat-stress associated production losses are less severe in beef cattle due to their lower metabolizable energy (ME) intake, metabolic rate and seasonal breeding. In beef cattle, there is a 0.4 kg/day average daily gain (ADG) depression for every 1°C increase in internal body temperature (Finch, 1986). St-Pierre et al. (2003) estimated heat stress losses of 2.4 billion US dollars across the entire beef sector in the USA. However, the authors do point to the limited data on reproductive

losses and heat stress related deaths. They determined the lost value of the national breeding herd to be 87 million US dollars p.a., with 282 million US dollars p.a. of lost value in the finishing herd (averaging to 1.5% annual income in all cattle rearing states). The very hot states such as Texas would expect to see an average growth reduction of 10 kg/head per year (with a range of 1.5 – 17 kg). There are qualifications on such economic assessments. Breed differences, acclimatization of animals, lag effects after a hot spell, and differing and inconsistent management practices limit the accuracy of these studies. Additionally, the utility of extra energy gained from increased digestibility is lost due to a 7 – 25% increase in maintenance requirements during warm conditions (Beede and Collier, 1986).

Heat waves can be a major cause of feedlot cattle fatalities. A mild heat wave in spring or early summer can be the most fatal since animals have not yet acclimatized to the warmer conditions (Nienaber and Hahn, 2007). There have been a number of heat wave events in the US over the last 20 years: 1992, 1995, 1999, 2002, 2006, 2009, 2011 and 2012. Many of these events have resulted in substantial mortalities in feedlots. For example, during 1995 there were 3,750 deaths in Iowa, and during 1999 there were 5,000 deaths in Nebraska (Brown-Brandl et al., 2006). The estimated losses from these events were 20-30 million US dollars for each episode, which approximated to 4000-5000 US dollars for every fatality (Mader et al., 2002). The catastrophic Californian heat wave of 2006 resulted in the death of 25,000 cattle and dairy and beef producers lost more than 1 billion US$ (Collier and Zimbelman, 2007). This event was followed by another heat-wave in 2009, which the death of thousands of cattle at an estimated loss of 20 million US dollars (USDA, 2010). The widespread heat wave of late July 2011 is reported to have killed 6,500 head of cattle in the states of Iowa, Minnesota and South Dakota. Data are not yet available for all the affected states. Lower but significant mortalities occurred in 2012 (Mader, 2012).

Records and assessments of Australian heat waves losses are not so readily available Beef cattle losses due to significant heat waves events have occurred in Australia since the early 1990, but not to the same extent as in the US. The major events and associated cattle deaths (where known) were:

- 1991 in Queensland (3,200),
- 2000 in Queensland and New South Wales (1,600),
- 2002 in South Australia,
- 2003 in New South Wales,
- early 2004 affecting New South Wales and Queensland (900 cattle), in late 2004 in South Australia,
- early 2006 in Queensland, and late 2006 affecting central New South Wales (Gaughan, 2008).

Whole of summer production costs are also under review. While the numbers and costs of cattle mortality due to a discrete heat event is relatively simple to calculate, total production losses over summers and on a national basis are difficult assessments. Sackett et al. (2006) estimated that Australian feedlots lose $16.5 million (due to reductions in animal performance) over summer. Mayer et al. (1999) developed an economic estimate of heat stress losses in NSW and Qld dairies. They found a general decline in milk production as Temperature Humidity Index (THI) increased and losses were greater for higher production dairies. This was influenced by adaptation since tropical dairies saw decreased milk yield at higher THI. On milk value alone, they estimated losses for a 100

head high producing farm was $12,000. The summer season also impacts on the animal's resilience to seasonal infection and disease. There are good correlations between the Temperature-Humidity Index and livestock deaths in USA and Europe (Dechow and Goodling, 2008; Stull et al., 2008; Vitali et al., 2009). Similar analysis for feedlots cattle has not been undertaken in Australia.

2.2 Current management intervention to ameliorate heat stress in cattle

The livestock industries have taken three approaches to moderate the impacts of hyperthermic conditions on intensively managed animals:

- improved environment,
- better preparation for heat events through specialised weather services, and
- adjustment of ration components during hot weather periods.

The dairy industry has engineered cooler environments by provision of shade and judicious use of location and time of water spraying and fans. The feedlot industry has done considerable work to develop and install shades and increase water supply. Due to the nature of the feedlot environment, sprays and fans are not ideal. The Panting Score combined with the Kayestone prediction service and the Risk Assessment Program has done much to assist the industry to manage heat waves event.

Based on research and their own experience, feedlot nutritionists have manipulated the buffering capacity, electrolyte balance and roughage: concentrate ratios of summer rations. These adjustments have met with success in some instances and not others, with no real understanding as to the basis of this inconsistency.

3 The Way Forward

3.1 Synopsis of the Review

3.1.1 Introduction

Over the last two decades, the Australian cattle feedlot industry has sought to be proactive in the management of heat load of animals in their charge to enhance and/or maintain welfare and productivity. ALFA and MLA have co-invested consistently in R&D in the development of management advice and tools for lot feeders. A number of drivers are encouraging both producers and researchers to focus on heat load management

- More extreme weather events and global warming
- Increased numbers of cattle in feedlots
- Increased productivity of cattle in feedlots and
- Increased societal oversight of animal welfare (Brown-Brandl et al., 2003).

Fatalities during heat wave events and the now well understood losses to production (particularly in the USA) indicate that anticipation and specific interventions can reduce much of the overt harm. The research effort into management tactics for severe events has yielded planning tools and action programs that have been put into practice by producers (e.g. MLA, 2006). The research has largely focused on gaining an understanding of animal response to high heat load (e.g. respiratory

dynamics), environmental management strategies (e.g. shade, water application), nutritional strategies (e.g. fibre, fat, betaine), tools for prediction of heat stress events (e.g. Kayestone/MLA prediction service; heat load index, risk assessment program (RAP)) and tools for assessing cattle responses (e.g. Panting Scores).

In Australia, mass mortalities due to heat wave events are now very infrequent; however, reduced production alone in feedlots over the summer season has been estimated to cost $16.5 M from reduced performance (Sackett et al., 2006). The consumer and market demands in the foreseeable future will place greater pressure on production systems to avoid thermal discomfort in livestock. Having dealt with management of animals through severe events, the industry is now in a position to address the cost to production of heat events to the 'survivors'. The major and most obvious contribution to productivity loss are decreased feed intake, the subsequent slower weight gain, and increased likelihood of infectious disease. Less obvious are the metabolic and immune system disorders.

Despite considerable R&D investment by the MLA (projects FLOT.307; FLOT.314; BFLOT.0343 & BFLOT.0345), little inroads have been made into the development of successful nutritional strategies to offset or ameliorate these losses. Many of the 'off-the-shelf' feed additives have been trialled with no or equivocal improvement to productivity or cattle comfort. These outcomes highlight the necessity of looking deeper into the altered physiology and metabolism of feedlot cattle over the summer period and during heat waves.

Feedlot cattle are predominantly young male-castrates cattle on high concentrate rations. The most commonly observed effects of increased heat load are reduced feed intake (resulting in reduced growth rate), reduction in feed efficiency during and after a heat event, and increased frequency of health-related issues especially Bovine Respiratory Disease. Further economic costs for the enterprise are incurred from higher management costs through increased cleaning of bunks, surveillance of animals, and disease. There is ongoing loss of productivity from the probable effects of heat stress on energy and nitrogen metabolism, with the hypoxic and oxidative damage to the rumen and gut, and the consequences of systemic inflammation adding to the accumulative losses.

3.1.2 Coping with heat load and systemic responses

The review has encompassed necessarily some of the expanding literature on responses to heat load in dairy cows, rodent models, and humans, and in doing so has highlighted the complexity of systemic responses of animals to hyperthermia. The overt characteristics of heat stress, reduction of feed, reduced appetite and lassitude are the accumulation of systemic endocrine, metabolic and inflammatory changes. A synopsis of the findings follows, and this is the basis of the development of the recommended R&D plan for management of heat stress for feedlot cattle.

3.1.2.1 Physiological responses

Much research effort in Australia and USA in particular has centred on improving and standardising meteorological measures and indices as a guide to predicting cattle responses and management needs. The best known of these are the Temperature–Humidity Index (THI), the Wet Bulb Globe Index (WBGI), the Black Globe Humidity Index (BGHI) and the Heat Load Index (HLI). The models do not include the physiological responses to heat load which are now well described: decreased feed intake, altered blood flow to skin, increased sweating and respiration rates, respiratory alkalosis,

increased body temperature, and reduced immune status. More recent research has attempted to find other indicators of responses, for example blood levels of heat shock proteins and acute phase proteins (haptoglobin). The problem with these examples is that as they are general stress indicators, they are responsive to a multitude of stressors. In practise, rectal temperature measurements respiration rate, and Panting Score are still the most common means of assessing heat stress in cattle. The Panting Score combined with the Kayestone prediction service and RAP has gone a long way to averting major fatalities at modern Australian feedlots.

3.1.2.2 Endocrine and metabolic changes in heat stress

Any stressor will redirect endocrine and metabolic processes toward maintenance of homeostasis and away from growth. The hypothalamus drives and coordinates the response through several signalling hormones, either working at the pituitary or directly on the target organ. The endocrine regulation behind the reduced feed intake observed during hot conditions in most mammalian species is poorly understood.

Some of the stress hormones show specific responses to heat stress. The adrenal gland hormones, aldosterone and the catecholamines show increased secretion into the blood. Aldosterone secretion is decreased in an effort to limit Na^+ conservation by the kidneys, since cattle need to conserve K^+, not Na^+. Blood levels of adrenalin and noradrenalin are increased in both acute and chronic heat stress. The source of these hormones in chronic stress is most likely the nervous system. The catecholamines encourage glucose and fatty acid production to supply energy to the organs.

The metabolic hormones are also affected. The thyroid hormones have strong influence over metabolic rate thus heat production. Not surprisingly, the blood levels of thyroid hormones are lowered in heat stress in an effort to reduce basal metabolic rate and heat production. Reports of altered plasma growth (GH) levels in response to heat stress are inconsistent. However, there is consistent and a slight decrease in plasma Insulin-like Growth Factor-1 which drives most of the effects attributed to GH. Most significantly, heat stress increases insulin production and responsiveness.

The reduced feed intake most commonly experienced during heat stress has clouded much of the research and interpretation of the endocrine and metabolic effects that can be solely attributed to heat stress. Elegant experimental work conducted with dairy cattle in climate chambers at University of Arizona (Tucson) are starting to give answers. The experiments used a pair feeding design whereby the feed intake of the heat-stressed animals is measured, and that amount then given to the thermoneutral controls the following day. As a result of these careful studies, we can now conclude that relative to pair-fed thermoneutral animals, heat-stressed cattle are hypoglycaemic with a 5-10% lower in blood glucose and do not mobilize fatty acids from their fat stores; even under adrenaline challenge. It appears that glucose is the favoured fuel in heat stress.

The contribution of reduced feed intake vs. heat stress alone to nitrogen (N) metabolism is still not understood. Increased plasma urea N and increased urinary excretion of urea N indicates reduced gut microbiota activity (increased liver ammonia detoxification) and/or increased liver use of amino acids from muscle. Additionally, heat stress is known to induce deleterious changes to the redox status, both systemically and localised within tissues in rodent models and humans. There is some evidence that is also the case in dairy cows. Little work has been performed on the redox status of growing beef cattle in heat stress.

In summary, the metabolic changes in heat stress cannot be explained by reduced feed intake alone. Heat-stressed ruminants fail to enlist the glucose saving mechanisms used by under fed animals; i.e. do not consume their fat stores and become slightly insulin insensitive. It is probable, that to supply the glucose required for maintenance, protein in muscle is being catabolised.

3.1.2.3 Inflammatory and gut changes in heat stress

The source of the oxidative changes and the role of fat tissues in heat stress is coming to light. White adipose tissue, especially visceral fat, is known to have endocrine activity. The two major hormones of fat are leptin and adiponectin: both have powerful effects on energy metabolism. In the first study of its kind, Morera et al. (2012) used a mouse model to compare chronically heat-stressed mice with mice maintained in thermoneutral conditions fed 80% rations. The major finding was that leptin is secreted by fat in heat-stressed mice unlike their controls. The main effects of increased leptin are decreased food intake, increased insulin sensitivity, increased glucose uptake and use. These actions agree completely with observations of cattle. Also, leptin is a pro-inflammatory hormone and promotes oxidative stress.

While the fat stores appear to have altered function, the gut is a site for dysfunction. There is now good evidence that the gut barrier function is disrupted in heat stress. The role of heat stress in rumenal and intestinal dysfunction in heat stress in cattle was first proposed by Cronjé (2005). The disruption to gut function and integrity is a consequence of reduced blood flow to the viscera during heat stress, as the blood is directed to the skin for cooling. Rat models, thus far, have shown decreased intestinal blood flow and lack of oxygen to the gut during heat stress which leads to damage and possibly death of gut cells.

As early as the 1970's, increased circulating levels of endotoxin has been associated with heat stress, and implied breach of intestinal barrier. Endotoxin, the lipid part of lipopolysaccharide component of the outer membrane of many gut bacteria, breaks off when the bacterial cell dies. It is very toxic when not confined to within the gut. If the liver cannot deal with the inflow of endotoxin from the damaged gut, endotoxemia results, causing a system-wide inflammatory response. The lack of oxygen in the gut and liver, due to the reduced blood flow, compounds the situation by deleterious changes to redox status, thus setting off more inflammatory responses. The conclusion of these new studies, yet to be done in cattle, is that of multiple inflammatory signals occurring in response to heat stress. The ensuing fever response only adds to heat load.

3.1.3 Current nutritional interventions for heat stress

Heat production in ruminants is in part due to the conversion of Metabolisable Energy to Net Energy. The physical and chemical work of eating and ruminating, rumen fermentation and finally gut and liver tissue metabolism, all contribute to the heat increment of any diet. Diet and its consequences for cattle, can differ by nutrient use efficiency, animal growth stage (efficiency of nutrient use is greater for maintenance than production) and composition of body gain (fat is deposited more efficiently than protein). Dietary components contribute to heat increment. In an above-maintenance situation, carbohydrates release 36-46% total energy as heat, in contrast to 25-45% of fats and 47-55% of protein discharged as heat. Thus, increasing the dietary ME during heat stress generally will not reduce lost productivity, due in part to 7-25% increase in maintenance requirements during high environmental heat load (NRC, 1981). Rumen protected dietary fats have

also been used as a means of increasing ME during heat stress, but despite their lower heat increment, the same principles apply. The addition of physically effective fibre to ensure sufficient chewing to induce salivary flows to buffer the rumen could be an important component for a 'heat stress' ration. The paradox is that fibre in itself has a high heat increment.

Respiratory alkalosis can occur in heat-stressed cattle as the increased respiration drives CO_2 from the blood, as the kidneys respond by removal of HCO_3^- from blood thereby reducing the levels of this important buffering ion in blood. The increased drooling during panting also results in less saliva (high in HCO_3^-) from entering the rumen, reducing the rumen's buffering capacity. The consequence is overt or subclinical ruminal acidosis, hence the 'standard practice' of providing a rumen buffer (HCO_3^-). Supplementation with buffering mineral salts in summer rations has had some success in partially overcoming reduced feed intake. Buffering capacity is closely aligned with dietary cation-anion levels. A more positive serum dietary cation-anion difference (DCAD) results in increased serum HCO_3^-. Potassium (K^+) is the primary osmotic regulator of water secretion from cattle sweat glands. As a consequence, dietary K^+ requirements are increased (1.4 to 1.6 % of DM). Dietary levels of sodium (Na^+) and magnesium (Mg^+) should be increased also as they compete with K^+ for intestinal absorption (West, 2002). Osmolytes such as betaine and glycerol have also been extensively studied with the aim of improving cellular hydration and integrity especially of the gut lining (mucosa).

Increased environmental heat of summer and associated severe heat events present feedlot managers with a difficult balancing act (Figure 3.1). They need to maintain productivity where the animal's energy and endocrine systems are diverted to a stress response, thus requiring careful management of diet and knowledge of consequences to altered diet and management regimes.

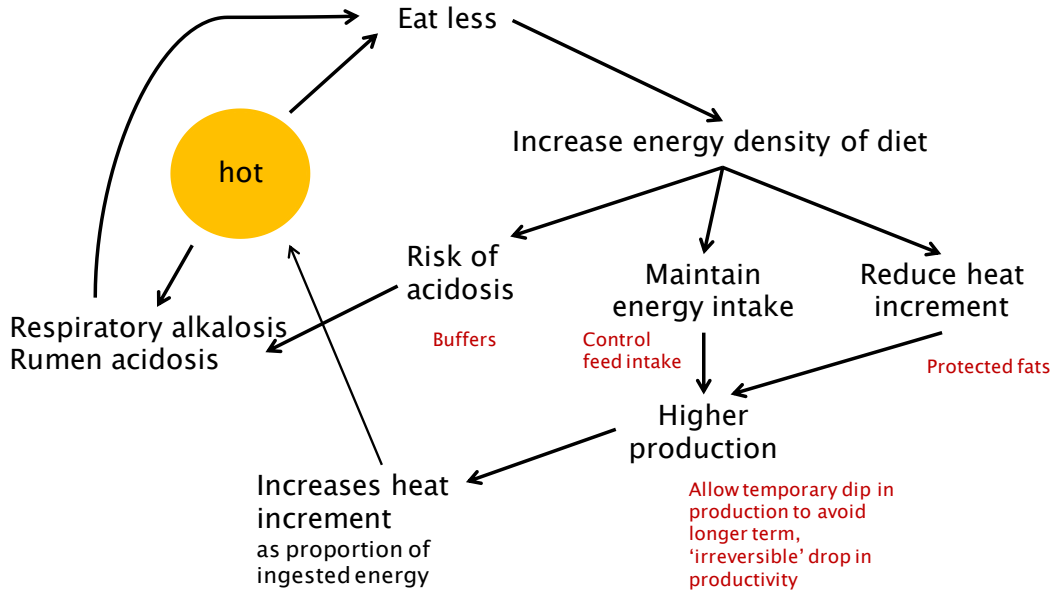

Figure 3.1. The balancing act.

Much research has been done for the application of dietary buffers, salts and altered grains: roughage ratios. If any research is to be done on these areas, it should be around the timing for making dietary changes during the summer period and over the whole course of a heat stress event.

However, the shifts in absorptive metabolism and nutrient partitioning are as significant (or more so) as the direct effects associated with feed intake and heat increment.

3.1.4 Feedlot industry research priorities for heat stress nutrition

The R&D priorities were developed during a workshop with industry representatives held in Brisbane over 4-5 September, 2012. The research team presented the conclusions of the literature reviews. The strong consensus was a need to have better nutritional knowledge for improved management of cattle through summer and during high heat load events. Two major questions arose:

Does the industry require a heat load nutrition program to ensure improved animal gut health, resilience and productivity over summer for an entire summer or for a shorter period within summer when risk of high heat load (heat stress) is highest (i.e. the January – February period)? (See Figure 3.2).

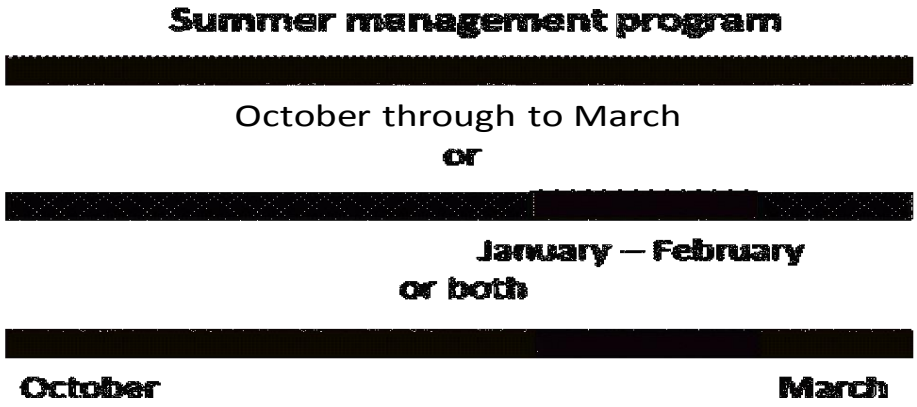

Figure 3.2. What period would require a 'summer management program"?

What are the most effective management and nutritional strategies for a forecasted heat event? To date the industry' primary concern has been to ensure animal survival. Much has been achieved and the industry is now in a position to consider strategies that will improve welfare and minimize gut and metabolic damage to animals and accelerate recovery. The need here is for staged implementation of a management plan that includes actions (such as different feeding times, increased water access) and dietary changes before, during the course of the heat event, and after the heat event (Figure 3.3).

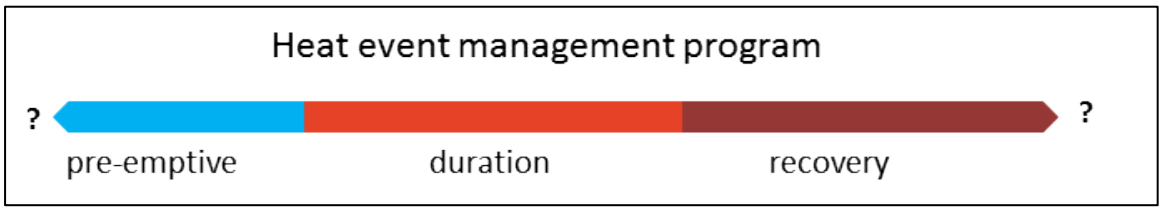

Figure 3.3 Proposed staging of a heat event management program

It was also emphasized that in conjunction with nutritional management programs that the industry also requires:

Improved accuracy and confidence in heat event forecasting (especially with rainfall events),

Better knowledge of the use and efficacy of pen management tactics such as surface wetting, bedding, etc.

Quantification of the loss ($ value per head) of production over summer and through major heat events.

A highly resourced and large program of research would, over 5 years, make much progress toward the needs delineated above. However, a realistic perspective requires some focus. All workshop attendees recognised that rumen and gut health was probably core to minimizing heat stress damage to feedlot cattle and addressing welfare and productivity. The proposed R&D program outlines a staged series of projects that places gut health as the focus of the program.

3.2 A recommended R&D plan

3.2.1 Gut integrity and inflammation in heat stress

While some research regarding gut health has been conducted in other mammalian species including dairy cattle, the extent of this problem in feedlot cattle is not known. Validation of gut damage and correlation with simple lab and animal observations (e.g. Panting score) would be central to the assessment of the efficacy of strategies and management interventions in and around heat stress events. Furthermore, since the investment in these studies would be considerable, every effort should be made to obtain as much biological understanding as possible, of these processes during high heat load and recovery.

Validation that gut damage and the ensuing inflammatory changes are occurring in heat-stressed feedlot cattle. Two approaches could be undertaken in parallel.

A pair feeding climate chamber experiment modelling a heat stress event (including the post-stress period), and contrasted with thermo-neutral controls.

Survey data with post-mortem samples from collaborating feedlots.

Validation that gut damage occurs as a consequence of underlying subclinical acidosis and normal summer conditions. Two approaches could be conducted in parallel.

A climate chamber experiment modelling moderate stress conditions on animals pre-conditioned to high concentrate rations.

Field experiment sampling from feedlot cattle on high concentrate rations over summer and winter periods.

The climate chamber experiments will guarantee intensive monitoring of physiological responses and intensive blood sampling for multiple analyses of metabolites, endotoxin, and metabolic and inflammatory hormones. These studies would provide valuable information regarding the status and dynamics of the inflammation, energy metabolism, and muscle breakdown. The field experiment sampling will be subjected to similar lab assays but collected less frequently and with fewer physiological observations. The post-mortem survey samples will be subjected to histological

analyses to assess rumen, intestinal, and liver damage. Records will be obtained of donor animals' condition, background and breed to correlate with tissue observations and any blood analyses.

3.2.2 Dietary additives

Several feed additives and ration adjustments have shown promise in some field trials but not in others. Furthermore, due to the nature of field experiments, only simple measures of responses (DMI, ADG, respiration rate, body temperature) were generally obtained. We suggest that future studies should also incorporate measures of animal (gut) health developed by the projects described above to provide new insights into mechanisms of action and, more importantly, to help clarify the circumstances when a nutritional intervention is more (or less) likely to be effective.

Initially candidate dietary additives or altered dietary regimes will be appraised for economical and feasible industry use (cost/unit; cost/animal; availability; price volatility; stability to storage etc). Those progressing through this filter will be subjected to climate chamber assessment where both traditional and newer measures of the cattle responses will be collected and analysed. Depending on these outcomes, the successful additives or ration change (e.g. **fibre type and content**) would go on to acute period summer pen trials and/or all of summer pen trials (Figure 3.4). A crucial component to this work will be to better target the use of a particular nutritional intervention (including additives) to the circumstances when it is most likely to be effective; i.e. before, during or after a heat event, or during prolonged exposure to heat (e.g. subclinical heat stress). We hypothesise that the apparent variability in responses to nutritional intervention are due, to some extent, to differences in the timing of the intervention relative to the accumulation (or loss) of a heat load imposed on the animals, and lack of understanding (or measures) of the health status of the animal.

Such trials could test the use, dosage and timing of:

- Limit or other programmed feeding strategies
- Increased effective fibre (fibre that stimulates rumination)
- Anti-inflammatory compounds
- Anti-oxidants that show promise in other systems
- Increased buffering.

3.2.3 Implementation of the program

We recommend that the R&D program commence with three approaches in parallel:

1. Intensive animal experiments, utilising climate chamber studies to define the impact of heat load on gut health (epithelial integrity) and tissue metabolism (e.g. preferred energy substrates, fats vs. glucose).
2. Dietary manipulations, including altered amounts of effective fibre can maintain a more suitable rumen environment through buffering from saliva without adding to the dietary heat increment.

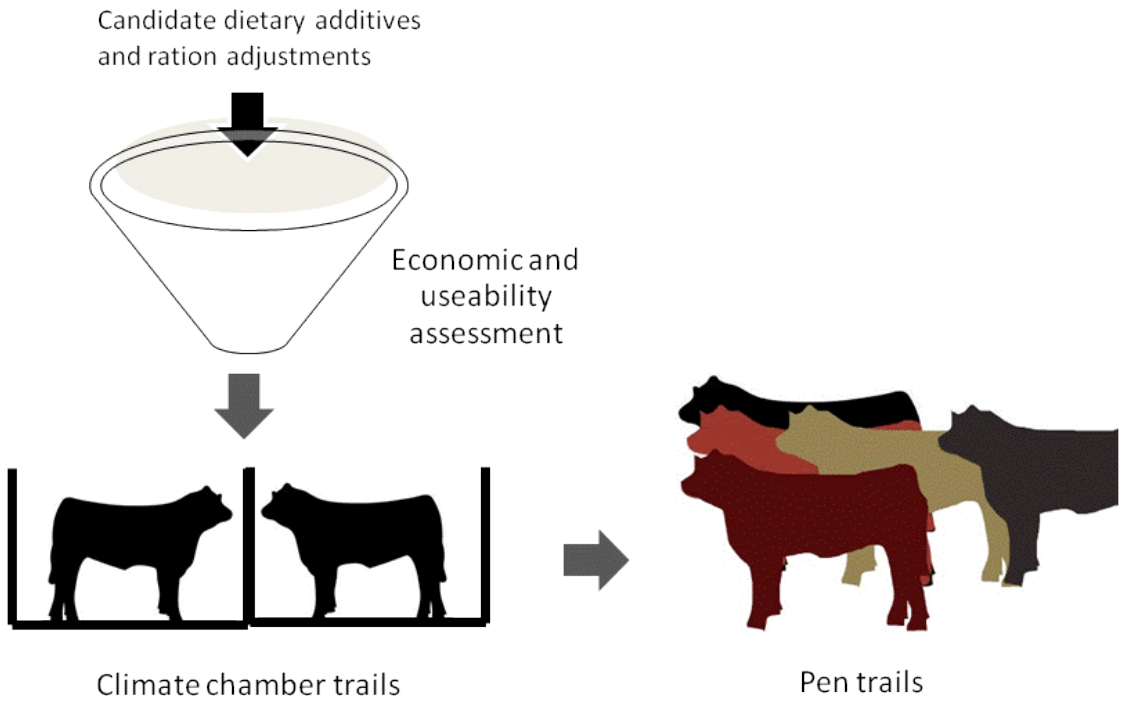

Figure 3.4

3. Surveys and tissue collection from feedlots through summer. Surveys of port-mortem material require enough summer seasons to collect statistically appropriate numbers of samples.

Following these studies, which will provide new knowledge on gut health and metabolic responses, we recommend climate chamber testing of dietary additives to specifically target gut function, inflammatory responses, and tissue energy metabolism, with the foci depending on the outcomes of the preceding experiments.

Testing of nutritional interventions under commercial conditions should commence as soon as the mechanisms of action are better understood. For management options to be tested in pen trial experiments, the judicious use of every summer season, preferably in different locations to cover a range of conditions, is required to ensure timely completion of the program.

4 Heat Load in Cattle

4.1 Brief overview of heat stress measures

Thermoregulation is complex and very dependent on environmental uptake of moisture; therefore, two components are required to ascertain responses to local climatic conditions: measurement of the meteorological change of a microclimate (ambient temperature, water vapour, solar radiation and convection), and the animals' response to that change. Researchers have considered a number of approaches to develop and refine proxies or predictors of heat stress or heat stress conditions. These attempts range from correlations amongst simple biological and meteorological parameters and composite measurements or indices, through to complex computer models of thermal balance (Hahn et al., 1992; Parkhurst, 2010; Turnpenny et al., 2000).

Studies of thermoregulation are further confounded by difficulties in replicating the operating environment in an experimentally-controlled situation. Climate-controlled rooms with temperature and humidity controls are useful but cannot replicate the solar radiation of a field environment (Finch, 1986). Furthermore, climate room conditions of constant temperature and humidity give different physiological responses to the field situation. Under the same parameters of ambient temperate and humidity, animals in indoor-housing conditions tend to have lower rectal temperature, lower skin temperatures and increased sweat rate. Also such facilities can accommodate a few animals only at a time further limiting the scope of these experiments. The following sections summarise the more commonly used weather and physiological indices applied to describing and managing heat stress in cattle.

4.1.1 Meteorological measures and indices

The impact of weather conditions on cattle has been extensively studied. Ambient temperature and air moisture content (usually expressed as relative humidity (%RH), although wet-bulb temperature and dew point are also commonly used) are the primary weather variables which affect an animal's ability to maintain core body temperature. However other variables such as direct and indirect radiation, and air speed can also have significant impacts. Furthermore, the duration of exposure and the intensity of conditions to which an animal is exposed will also affect thermoregulatory ability. The direct effects of weather on an animal can be further modified by: animal factors – such as age, body condition, coat colour, hair density and length, health status, level of production, acclimatization, and behaviour (e.g. shade seeking), and management factors – such as housing and nutrition. It is clear that there is a complex interaction between the animal and its climatic environment (see Gaughan et al., 2009a). In an attempt to simplify these interactions thermal indices have been developed.

The use of thermal indices as a measure or predictor of heat stress on cattle was first investigated in the 1940's (e.g. Rhoad, 1944). However, it wasn't until the early 1960's that a livestock specific (dairy) index was developed (e.g. Johnston et al., 1962). Since then a number of indices based on climate variables have been developed for a range of livestock and poultry, and as these have been have given good coverage of this topic, only a brief description of the common indices are presented below (Hahn et al., 2009; Gaughan et al., 2012).

The predominant index incorporates ambient temperature and relative humidity, hence the term Temperature Humidity Index or THI. The THI has been a long standing and broadly applied indicator of thermal comfort (Collier and Zimbelman, 2007). It is a measure of the impact of ambient temperature on animals and was originally devised for indoor application. THI is calculated as:

$THI = T_{air} + 0.36T_{Dewpoint} + 41.5.$

The minimal inputs for the THI do make for easy use in most environments and are useful for retrospective studies in most regions since temperature and humidity data has been collected over many decades in most parts of the world. Notably, the THI does not account for solar load, wind speed or animal difference thus many variants have arisen to rectify this shortfall. For example, the Black Globe Humidity Index (BGHI) incorporates the contribution of solar radiation and has been reported to perform better in open environments although closed environments offer a multitude of microenvironments (Buffington et al., 1981; Collier et al., 2006a).

In its application to heat stress in dairy cows and beef cattle, a THI < 72 is considered thermoneutral; a THI ranging over 72-79 induces mild heat stress, whereas moderate and severe stress is caused by THI ranging between 80-89 and 90-98, respectively (Armstrong, 1994). However, Bilby et al. (2008) suggested a reconsideration of these classifications given that they were developed from retrospective meteorological and milk yield analyses of 1950-60's dairy herds which were producing an average of 43 L per day. Since the mid 1990's there have been attempts to address the limitations of the THI by developing indices that use multiple climatic and animal variables (Eigenberg et al., 2005; Gaughan et al., 2008a; Mader et al., 2010).

The Wet Bulb Globe Temperature (WBGT) is a composite measure that estimates the combined effect of air temperature, humidity, wind speed (wind chill), and solar radiation. Three physically measured parameters contribute to the equation:

$$WBGT = 0.7 \times T_{NWB} + 0.2 \times T_{BG} + 0.1 \times T_{ambient}$$

The Natural Wet Bulb temperature (T_{NWB}) records the temperature as affected by evaporation, humidity and convection by a thermometer with its bulb covered with a wet cotton sock supplied with distilled water (BoM, 2011). The Black Globe Temperature (T_{BG}) is measured by a 150 mm black globe with a thermometer located at the centre. T_{BG} represents the integrated effects of radiation and wind. $T_{ambient}$ is air temperate (measured in the shade).

The most recently developed indicator is the Heat load Index (HLI) was specifically developed for determining heat stress levels in beef cattle production (Gaughan et al., 2008b). It is currently utilised by the Australian feedlot industry (MLA, 2006). The HLI is determined from T_{BG}, relative humidity and wind speed. Depending in actual T_{BG}, one of two equations is used:

$$HLI(T_{BG>25}) = 8.62 + (0.38 \times \text{relative humidity}) + (1.55 \times T_{BG}) - (0.5 \times \text{wind speed}) + e^{2.4 - \text{wind speed}}$$

$$HLI(T_{BG>25}) = 10.66 + (0.28 \times \text{relative humidity}) + (1.3 \times T_{BG}) - \text{wind speed}$$

As with the THI, several thresholds have been nominated as a guide to heat load in cattle: HLI<70 is thermoneutral; HLI 70.1-77.0 is warm; HLI 77.1-86.0 is hot; HLI 86.1-96.0 is very hot and HLI>96 is extreme. However unlike previous models where the thresholds are set for all animals, the HLI thresholds will vary with animal type, health status, level of production and numerous other parameters (Gaughan et al., 2008b).

4.1.2 Body temperature measures

Internal body temperature is a very good predictor of animal wellbeing and comfort (Shearer and Beede, 1990) and has been used for diagnosis of onset of infections, stress and oestrus (Davis et al., 2003). It is overall the best indicator of the thermal status of cattle (Gaughan et al., 2008a). Most of the heat production in cattle is generated from the major organs (heart, liver, kidney, gastrointestinal tract and brain) with only 3-8% generated by fermentation in the rumen and gut. In cattle, the normal internal body temperature range is 38.0-39.5°C with a distinct circadian rhythm of 1°C daily variation regardless of feed intake or climatic conditions. Under thermoneutral conditions, the daily maximum tends to occur in late evening with the daily minimum occurring in late morning (Hahn, 1999). Body temperature of cattle is usually measured as rectal temperature. However, vaginal temperature, internal body temperature, temperature, tympanic (ear) temperature and

rumen temperature are also used. Body surface temperature and orbital temperature has also been measured using infrared thermometers and thermography.

Traditionally body temperature was obtained using mercury thermometers that were placed rectally in restrained animals in an effort to obtain a spot measure of core temperature. Using this method, rectal temperature was usually obtained once a day, every couple of hours and even every couple of days. Obviously infrequent collection of body temperature does not allow for a full understanding of the thermal dynamics within an animal. The advent of data loggers, radio transmitters and associated technology has allowed for real-time continuous recording of body temperature in both controlled (animal house) and field studies. Currently, rectal temperature is measured by a probe attached to a data logger and is useful for continuous readings for 5 – 7 days, after which time it may provoke local tissue inflammation depending on season (Davis et al., 2003). Rectal temperature is normally 0.2-0.6°C higher than internal body temperature and lags by 4-5 hours behind changes in ambient temperature (Brown-Brandl et al., 2003; Davis et al., 2003). While Davis et al. (2003) calculated an average correlation of 0.57 for rectal temperature and internal body temperature continuously monitored over 24 hours, correlations as high as 0.92 have been recently achieved (Gaughan et al., 2010b), but interestingly rectal temperature shows poor correlation with ambient temperature (Scharf et al., 2010).

Internal body temperature is determined by placement of a temperature logger (or transmitter) in the peritoneal cavity (usually between the internal abdominal (abdominal oblique) muscle layer and the peritoneum at the right side flank, behind the rib cage). This temperature measure is considered the “gold standard” but there are clear disadvantages in the utility of a surgically implanted device in terms of cost, labour and recovery time, and the limited number of animals that can be assessed. Temperature measurements at several other sites are used as a proxy for internal body temperature and each is characterised by advantages and disadvantages. These idiosyncrasies must be understood since placement of thermometers at different sites gives different readings leading to different interpretation of the relationship between body temperature and ambient temperature (Scharf et al., 2011). Four sites not requiring surgery have been used to collect proxy data are the rectum, ear canal and rumen. Details and notes on several of the devices are given in Appendix 8.2.

Tympanic temperature is collected by insertion of a thermistor probe into the ear canal. It is the easiest temperature measurement to collect in a production setting, being non-invasive, easily inserted and easily retrieved post-slaughter (Davis et al., 2003). Furthermore, it is affixed to a low value part of the carcass but it must be removed after 7-10 days to prevent irritation. Tympanic temperature is normally 0.2-0.5°C higher than internal body temperature and experiences a diurnal variation of 0.5-1.2°C as well as natural oscillations of approximately 30 minutes and 0.1-0.3°C that appear to be linked to feeding and activity. It is very responsive to changes in ambient temperature. Similar to rectal temperature, Davis et al. (2003) found that 24-hour continuously monitored tympanic temperature had an average correlation with internal body temperature of 0.58. In the same experiment, rectal temperature and tympanic temperature achieved correlations for 0.75. However, minimum daily rectal temperature and tympanic temperature showed poor correlation (0.33 and 0.35) with minimum daily internal body temperature (Davis et al., 2003). There appears to be no or a very small consistent difference between rectal temperature and tympanic temperature (Prendiville et al., 2002; Davis et al., 2003).

Rumen Temperature is collected using intra-rumenal device that transmits data to an external receiver. A clear advantage of a rumen device compared to most other in- or on-animal thermometers is the decreased labour costs from its simple installation in the animal. Rumen temperature varies from 40°C at the top and middle of rumen to 38.5°C at the base; therefore, consistent positioning of the device amongst animals is important (Davis et al., 2003). Beatty et al. (2008) found that the devices they used were all confined to the dorsal sac of the rumen. As with internal body temperature, rumen temperature follows a natural circadian rhythm with an average 1°C variation (Beatty et al., 2008). Rumen temperature is generally higher than internal body temperature by up to 2.2°C, mostly likely due to heat of fermentation generated by the gut microbiota. Rumen temperature exhibits dramatic temperature drops and recoveries mostly likely associated with ingestion of water (Beatty et al., 2008) although this could be useful if it allows drinking events to be determined. Every 2°C drop in rumen temperature caused by a dinking episode induced a 0.1°C decrease in internal body temperature which persisted for 10-20 minutes after these episodes. Water intake can affect rumen temperature for up to 3½ hours post-drinking depending on volume and water temperature (Rose-Dye et al., 2011). Rumen temperature is 0.13-1.25°C higher than rectal temperature and highly correlated with rectal temperature but the correlation can be affected by external factors such as season, lactation status and housing conditions (Bewley et al., 2008; Rose-Dye et al., 2011).

Skin Temperature has much variation from site to site due to blood flow (Davis et al., 2003). Scharf et al. (2010) found that when averaging the skin temperatures taken from 5 different sites, skin temperature gave good to very good correlations with ambient temperature and respiration rate (r^2 = 0.93 and 0.74 respectively) but poor correlation with rectal temperature. Use of infrared thermography to determine has also been proposed as a means of monitoring surface temperature and the temperature at the orbit of the eye (orbital temperature) in larger numbers of animals in production environments (Schaefer et al., 2012). The technology is not invasive and animal handling is not required. The equipment can be installed in a diversity of environments including pens and paddocks and can be automated. Surface temperature as measured by infrared thermography is highly correlated with respiration rate and is informative of the animal's microenvironment (Collier et al., 2006a). It has been successfully applied in monitoring of changes during bovine respiratory infection in calves (Schaefer et al., 2007; 2012). Clerc et al. (2012) working with beef cattle in North Queensland found good correlations with rectal temperature and orbital temperature as measured by infrared thermography. Under these conditions, orbital temperature was generally 1-2°C less than rectal temperature. The caveat is that orbital temperature does interact more rapidly with the immediate environment but is less affected than surface temperature measured at any number of sites on the body (Clerc et al., 2012).

In the dairy environment, both vaginal temperature and freshly expressed milk temperature have been used successfully as proxies for internal body temperature. Vaginal temperature is measured and recorded by intravaginal probes, allows 24 hour monitoring of body temperature and numerous studies have been undertaken measuring vaginal temperature (Kendall et al., 2006; Tucker et al., 2008; Schütz et al., 2009; Vickers et al., 2010; Burdick et al., 2012). Vickers et al. (2010) compared rectal temperature to vaginal temperature in dairy cows and reported correlations for fresh cows (n = 1,393; r = 0.81) and for peak-lactation cows (n = 556; r = 0.46) while Burdick et al. (2012) reported a correlation r = 0.98 for rectal temperature to vaginal temperature.

4.1.3 Respiration rate and Panting score

In response to heat stress, ruminants increase their respiration rate as one means of reducing heat load. Respiration rate responds over 1½ to 2 hours to increasing ambient temperature although the respiration rate will vary between individuals and across breeds during heat exposure (Scharf et al., 2010). Respiration rate appears to be a reliable and practical visual indicator of heat stress, if monitoring is performed in reasonable proximity to the animals, or automated. Although respiration rate is routinely used as an indicator of thermal status it is difficult to obtain under commercial conditions. The panting score (PS) system was designed as a visual assessment of the thermal status of cattle (Mader et al., 2006; Gaughan et al., 2008a). The PS system uses an 8 point score (0, 1, 2, 2.5, 3, 3.5, 4 and 4.5) where PS = 0 indicates no elevation in respiration rate (no thermal stress) and a PS = 4.5 indicates severe thermal stress (open mouth, tongue fully extended, head drooped, rapid laboured breathing) (Table 4.1). A PS = 2 (closed mouth, drooling, elevated respiration rate) indicates that the animal is under moderate heat load. A PS = 3 (open mouth, tongue extended, drooling, further elevation of respiration) is an indicator of high heat load. On days of moderate to high heat load cattle will move between a panting score of 2 and 3 on a regular basis. Therefore, observation is required more than once during the day. The optimal times are early morning (approximately one hour after sun rise), midday (1200 to 1400 h) and late afternoon (e.g. one to two hours before sunset). Panting score has a strong correlation with body temperature (Figure 4.1), and therefore is a good indicator of body heat load (Gaughan et al., 2012), and will allow for identification of heat-tolerant animals (Figure 4.2).

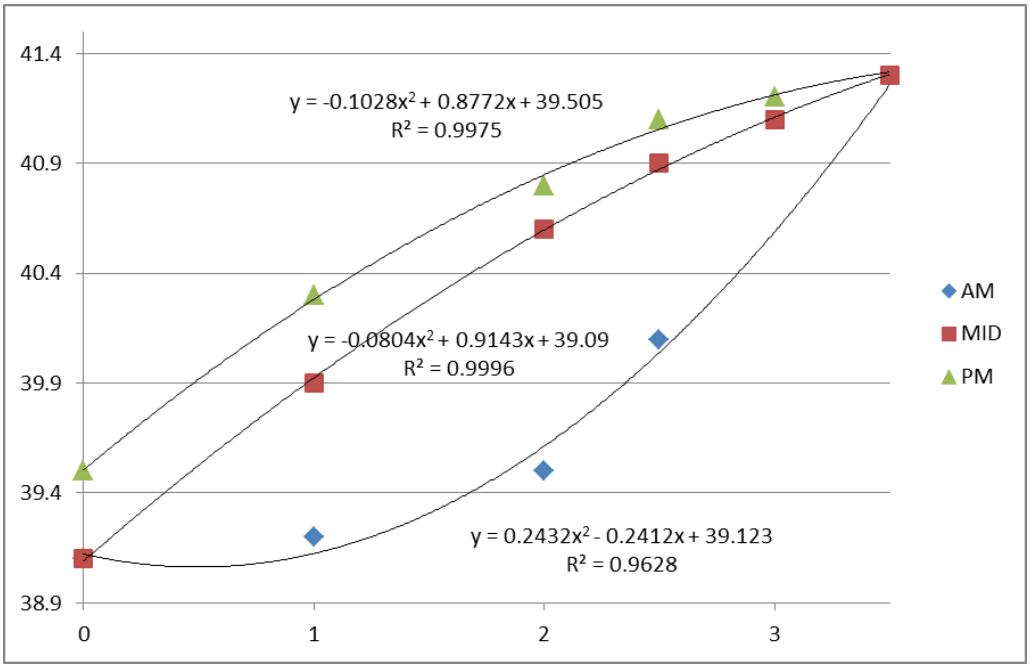

Figure 4.1. The relationship between panting score and core body temperature for Angus steers housed in a feedlot over summer at 0600 h (AM), 1200 h (MID) and 1600 h (PM) (Gaughan et al. 2012).

Table 4.1 Assessment of Panting Scores

Score	Description
0	No panting
1	Slight panting, mouth closed, no drool, easy to see chest movement.
2	Fast panting, drool present, no open mouth.
2.5	As for 2, but occasional open mouth panting, tongue not extended
3	Open mouth and excessive drooling, neck extended, head up.
3.5	As for 3, but with tongue out slightly and occasionally fully extended for short periods.
4	Open mouth with tongue fully extended for prolonged periods with excessive drooling. Neck extended and head up.
4.5	As for 4, but head drooped. Cattle “breath” from flank. Drooling may cease.

(Adapted from Mader et al. (2006) and Gaughan et al. (2008a))

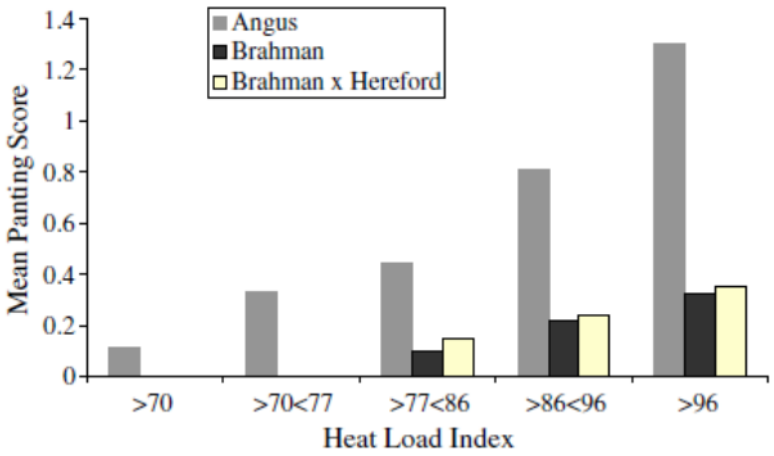

Figure 4.2. Mean panting scores of Angus, Brahman and Brahman x Hereford steers with access to shade under increasing heat load (Gaughan et al., 2010a).

4.2 Physiological responses

Most mammals have a number of physiological and endocrine mechanisms that can be utilised to counter increased heat load, but not all coping mechanisms are mobilised at the same time. The basic thermoregulatory strategy of mammals is to maintain internal body temperature higher than ambient temperature through four means of heat dissipation; three sensible routes which rely on a thermal gradient, and one means of insensible heat evaporation where heat loss depends on a water vapour gradient. If skin temperature is greater than 35°C, the temperature gradient is large

enough to allow for heat dissipation by all four routes. If ambient temperature is close to internal body temperature, evaporation is the only means of cooling if humidity allows (Collier et al., 2006a)

4.2.1 Body temperature responses in cattle

Knowledge of the relationship between the circadian change in body temperature and the thermal environment is needed to increase our understanding of maintenance requirements and limitations to productive performance (Araki et al., 1984), as well as animal welfare. Likewise, such information is essential for the evaluation of the benefits of any environmental modification (Igono and Johnson, 1990). However, body temperature is usually difficult to obtain under field conditions.

In earlier studies, body temperature of cattle was measured at selected times of the day (Bond et al., 1957; Mendel et al., 1971; Bond and McDowell, 1972; Thompson, 1973; Morrison and Lofgreen, 1979). Even though it was known that cattle exhibit a diurnal variation in body temperature, which reflects a combination of the thermal and physiological status of the animal (Wrenn et al., 1961; Simmons et al., 1965; Bianca, 1968; Berman and Morag, 1971; Scott et al., 1983). More recently the use of data loggers and wireless radio frequency transmitters have allowed continuous recording of body temperature of free ranging cattle at intervals of a few minutes for periods of a few days (usually less than 10 days; Davis et al., 2003; Mader and Kreikemeier, 2006; Gaughan et al., 2009a), to periods of weeks and months (Lefcourt and Adams, 1996; Brown-Brandl et al., 2005; Gaughan et al., 2010b) (Figures 4.3 and 4.4).

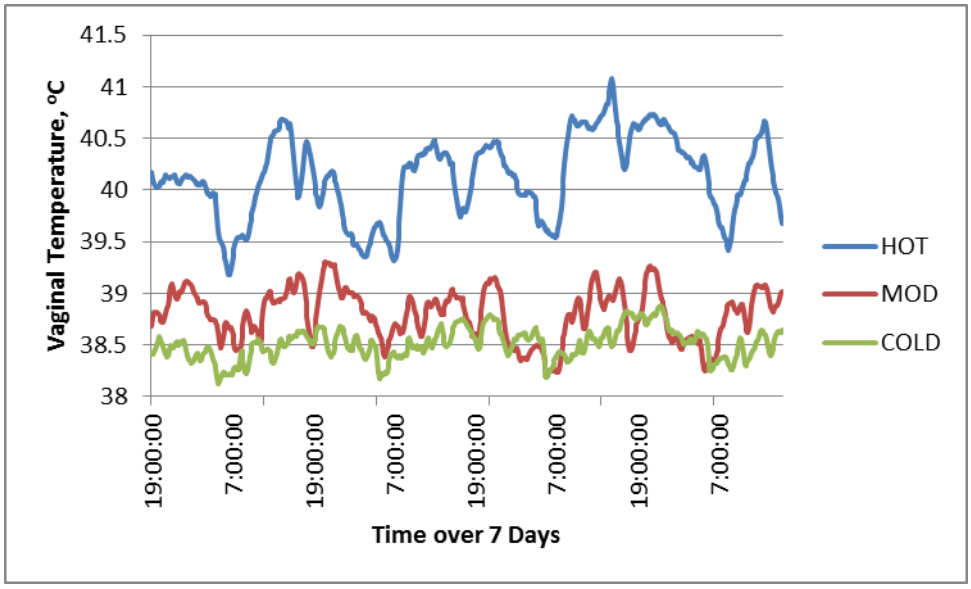

Figure 4.3. The vaginal temperature of dairy cows over 4 days during mid-summer (HOT), early summer (MOD) and winter (COLD) (Gaughan and Lees, unpublished).

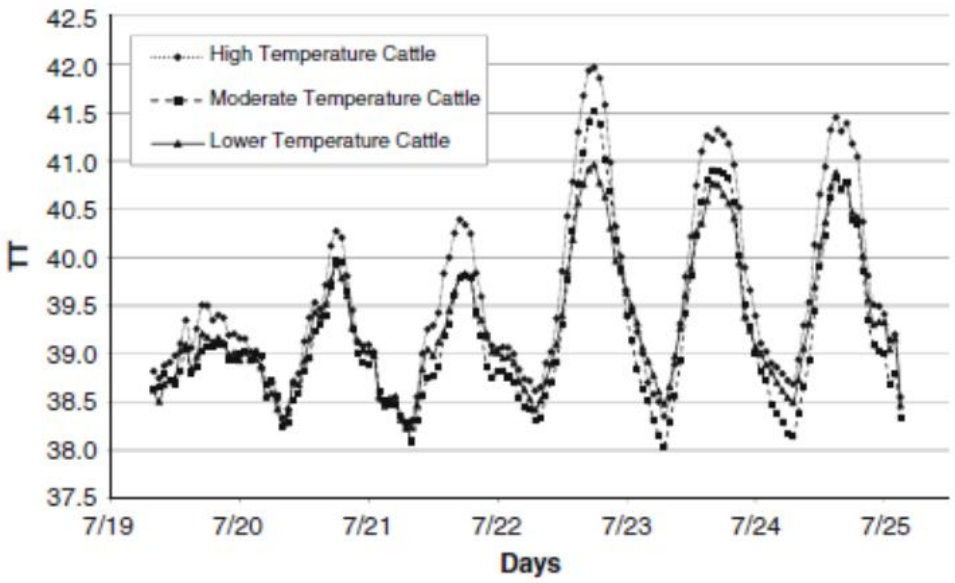

Figure 4.4. Tympanic temperature (°C) pattern in cattle exposed to summer conditions (Mader et al., 2010).

Core body temperature of cattle exposed to hot conditions lags ambient temperature by 1 to 5 hours (Hahn et al., 1999; Mader, 2003; Brown-Brandl et al., 2005), as opposed to an 8 to 10 hour lag under thermoneutral conditions (Hahn, 1995; Holt et al., 1998). A similar delay in peak internal body temperature was noted in beef cattle exposed to constant heat stress under controlled conditions (Zhang et al., 1994). In addition, daily mean and amplitude values for internal body temperature are increased during heat stress (Hahn, 1995). For some cows body temperature may even increase during the night, which increases the gradient for heat transfer to the environment (i.e. enhanced cooling) (Scott et al., 1983). Cattle exposed to an increasing ambient temperature (above 25.6°C) exhibited a linear increase in core body temperature (Lefcourt and Adams, 1996). The increase in body temperature at maximum ambient temperature was a rise of 0.085°C in core body temperature per °C increase in ambient temperature, above an ambient temperature threshold of 25.6°C. In addition, sharp peaks in core temperature were seen late evening (~2200 h), long after the daily decrease in ambient temperature.

The impact of high ambient heat load on cattle can be reduced if there is adequate shade (Mitlöhner et al., 2001; 2002; Gaughan et al., 2010a; Sullivan et al., 2011) (Figure 4.5).

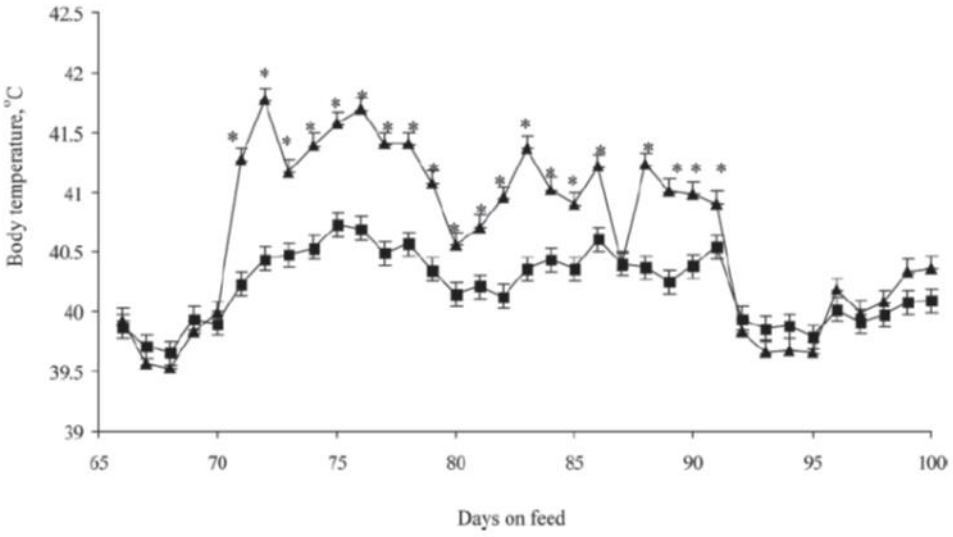

Figure 4.5. Maximum body temperature of feedlot steers with (■) and without (▲) access to shade prior to (day 66 to d 70), post (day 92 to day 101) and during (day 71 to 91) a 21 day heat wave (Gaughan et al., 2010b).

4.2.2 Evaporative cooling from the skin

In cool conditions, the rate of heat loss through evaporation from skin and the respiratory system is similar. When conditions are hot, evaporation from the skin becomes the dominant means of cooling, accounting for 80% of moisture loss; the remainder being lost from respiratory system (Bianca, 1965). Animals with efficient sweating capacity and responsiveness will maintain a lower respiration rate, and heat adapted animals are able to rapidly increase evaporative heat loss to stabilise internal body temperature (Finch, 1986). Tropically-adapted animals show a clear reliance on sweating rather than increased respiration rate. For example, *Bos indicus* responds to increasing ambient temperature with exponentially increasing sweat rate.

Sweating rate of cattle is not easy to measure under most conditions (see Schleger and Turner, 1965). Finch et al. (1982) reported that the relationship of the sweating response to mean rectal temperature was negative. Thus, the sweat rate response could be a good indicator of the thermoregulatory ability of the animal. Finch et al. (1982) also reported that, between animals within breeds, the sweating response was negatively correlated with metabolic rate. This suggests that cattle with high sweat rate (good heat adaptation) may have lower metabolic potential may be associated with lower rates of tissue synthesis rates and productivity. Gaughan et al. (1999) found a positive response between sweat rate and rectal temperature when steers were exposed to hot conditions, but no relationship under thermoneutral conditions.

There is however considerable variation in the literature in regards to breed and sweat rate (Allen et al., 1970; Pan et al., 1969; Amakiri and Onwuku, 1980; Finch et al., 1982). Generally the sweat rate of *Bos indicus* cattle is greater than for *Bos taurus* cattle when exposed to high heat load (Schleger and Turner, 1965). However, Gaughan et al. (1999) reported little difference in sweat rate between Brahman (171 g/m^2/h) and Hereford (175 g/m^2/h) steers that were exposed to THI > 90. Sweat rate of Brahman x Hereford steers exposed to hot conditions was 221 g/m^2/h (Gaughan et al., 1999)

suggesting a heterosis effect (Schleger and Turner, 1965). Differences in sweat rate between heat tolerant *Bos taurus* cattle (Romosinuano) and a non-tolerant breed (Angus) were investigated by Scharf et al. (2010). They reported that sweat rates were greater in Angus than for Romosinuano under thermoneutral conditions. Sweat rate increased more than four-fold during exposure to high heat load, followed by reduction after 7 days. Even after acclimation to high heat load the Angus exhibited the greater sweat rate (Figure 4.6).

Nay and Hayman (1956) reported differences in the location of sweat glands and density of sweat glands between *Bos indicus* and *Bos taurus* cattle. They found that *Bos indicus* cattle had larger and more numerous sweat glands than *Bos taurus* cattle. The sweat glands of *Bos indicus* cattle were more numerous on the mid-side than on the dewlap, and closer to the surface than for *Bos taurus* cattle. They suggest that these differences mean that the peripheral blood vessels on the dewlap are cooled by evaporation of sweat running down the dewlap.

The effectiveness of sweating is also dependant on coat type. If moisture is captured in the hair then the efficiency of sweating is reduced. However, there does not appear to be a breed effect on sweat rate (Allen et al., 1970), even though hair samples showed differences between breeds. Similar sweating rate between breeds does not necessarily indicate a similar cooling effect.

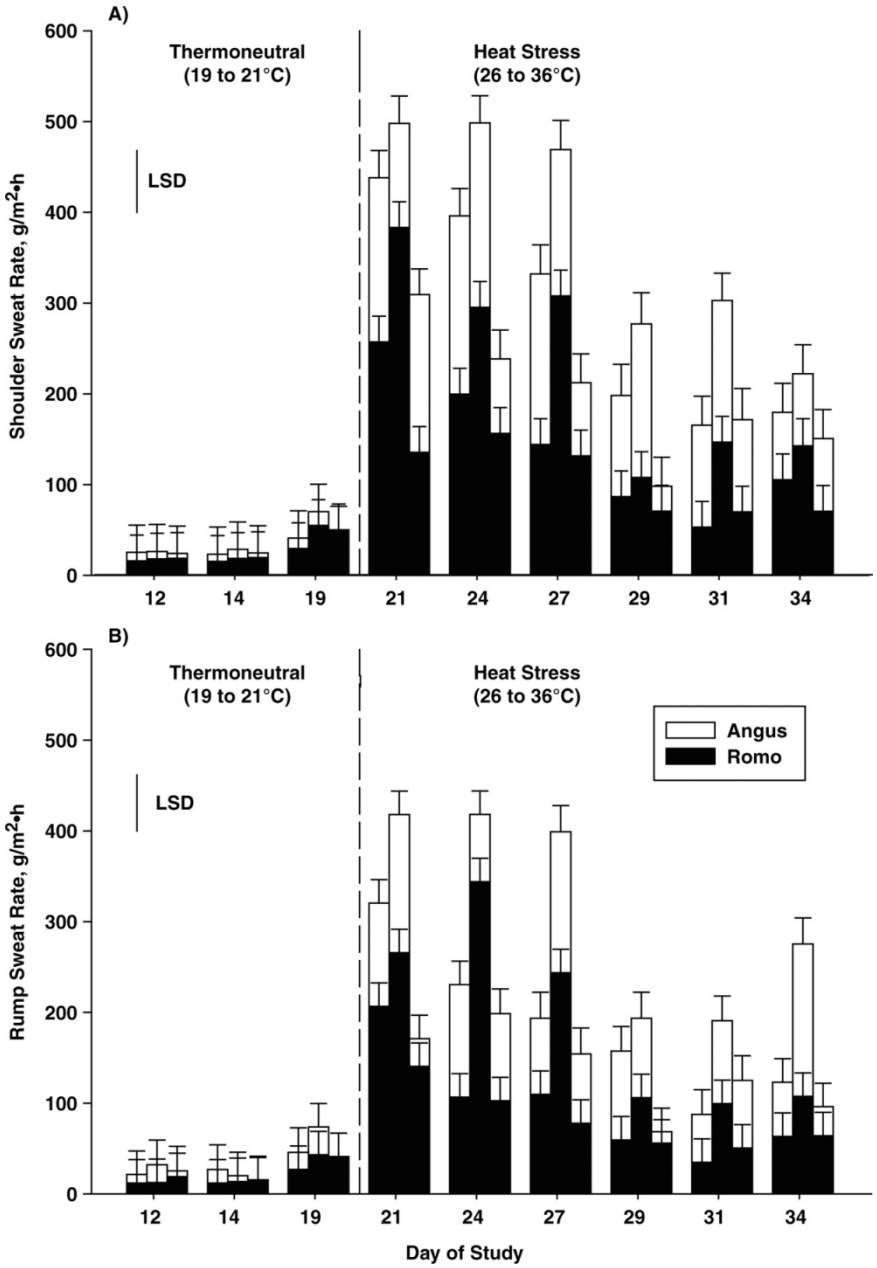

Figure 4.6 Mean skin sweat rate of shaved shoulder (A) and rump (B) skin sites for Angus and heat-tolerant Romosinuano (Romo) cattle on selected days before heat exposure (i.e., pre day 20) and during heat stress (i.e., post day 20). The vertical line on top of each column is +1 SE, and the vertical LSD line is for $P < 0.05$. (Scharf et al., 2010)

Variation in sweating rate is due to a combination of factors such as: the site of measurement on the animal, breed, whether the animals are acclimatised to hot conditions, climatic conditions prior to and during measurement, time of measurement, whether cattle are inside or outside (radiation effects), closeness to other cattle and availability of drinking water.

4.2.3 Evaporative cooling from increased respiration rate

The first observable reaction of cattle exposed to environmental temperatures above their thermoneutral zone is generally an increase in respiration rate (Figure 4.7). This enables increased

heat dissipation by evaporating moisture from expired air, and this can account for up to 30% of total heat dissipation from the animal (McLean, 1963).

It is generally true for all classes of cattle (beef or dairy, young or old) that respiration rate will increase with increasing ambient temperature (Allen, 1962; Webster, 1973; Young, 1975). However, the basal respiration rate of cattle exposed to thermoneutral conditions and the rate of increase when they are exposed to temperatures above thermoneutral are dependent on many factors, such as age, genotype, sex, level of nutrition, body condition, level of performance, activity, reproductive status, and modifying thermal conditions (wind, radiant heat load, wetting). Basal respiration rate, for example, decreases as dairy cattle get older (Badbeldin et al., 1951; McDowell et al., 1955); this is likely related to decreases in body temperature and heat production as the animal matures.

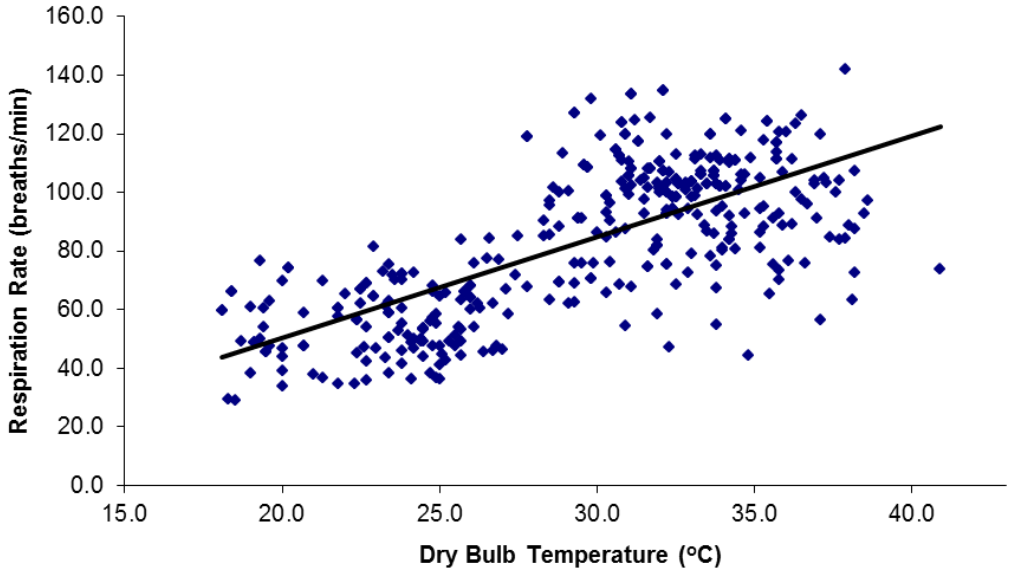

Figure 4.7. The relationship between respiration rate (RR; bpm) and dry bulb temperature (DBT; °C) (RR = -18.4 + 3.4DBT; r^2 = 0.4963, P <0.0001) (Gaughan *pers.comm*. 2012).

A similar finding was reported by Kibler (1957) for Brahman and Shorthorn calves up to about 14 months of age. Under similar climatic conditions older dairy cows usually have a basal respiration rate of 15 to 30 breaths per minute (bpm) (Benezra, 1954), while calves and beef cattle typically have a basal respiration rate of 30 to 60 bpm (Kabunga, 1992; Hammond et al., 1998). Prior exposure to elevated air temperature may also be a modifying factor (Spiers et al., 1994). Exposure of non-adapted animals to elevated ambient temperature is particularly challenging for the animal and this is reflected in increased respiration rate compared to adapted animals (Young and Degen, 1981).

Respiration rate is principally influenced by ambient temperature, thermal radiation (including solar radiation), relative humidity, and wind velocity. Of these variables, ambient temperature has been identified as the most important (Hahn et al., 1997). However, the relationship between respiration rate and ambient temperature is complex. Curvilinear (Gonyou et al., 1979), quadratic (Gaalaas, 1945; Robinson et al., 1986; Spain and Spiers, 1996; Hahn et al., 1997), and sigmoid relationships

(Kibler and Brody, 1954; Worstell and Brody, 1953) have been reported. Furthermore, increased respiration rate may lag behind the increase in ambient temperature. Significant correlation coefficients for lags ranging from zero to four hours have been reported (Hahn et al., 1997). During exposure to chronic hot conditions (over 4 days of exposure), the highest correlations were generally for respiration rate lagging ambient temperature by two hours. For acute exposure to conditions (first couple of days of exposure), observed overall lags tended to be slightly longer (typically two to three hours) primarily a result of the delayed respiration rate recovery at night. There was no relationship between respiration rate and ambient temperature during exposure to thermoneutral conditions.

A threshold air temperature for increases in respiration rate in *Bos taurus* beef cattle was found to be 21.3°C, with the slope of the linear relationship between respiration rate and ambient temperature above this point being 4.1 bpm per 1°C increase in ambient temperature (Hahn et al., 1997). This is lower than the 24°C to 25°C threshold for the increase in internal body temperature and associated decrease in feed intake reported by Scott et al. (1983) and Hahn et al. (1990; 1992). Likewise, the ambient temperature threshold for increased respiration rate is below that found to increase water intake (NRC, 1981). In general, respiration rate of steers can increase from non-stress levels of 30 to 60 bpm to a maximum rate of 160 to 200 bpm within one hour during both acute (two to three days) and chronic (seven to eight days) exposure to high ambient temperature. However, peak respiration rate is greater for acute compared to chronic exposures (~170 vs. ~140 bpm). It is likely that an adaptive respiration rate response to ambient temperature exists (Hahn et al., 1992). Black Angus cattle exposed to 32.2°C for seven days followed by a 20-day recovery period, exhibited a lower respiration rate to the same range of ambient temperature when tested a second time (Spiers et al., 1994). It has been observed in chamber studies that cattle which have had prior exposure to hot conditions (previous day) tend to increase respiration rate at lower ambient temperatures and at a faster rate than those without prior exposure (Gaughan, unpublished). This may be part of the adaptation/acclimatization process when cattle are exposed to hot conditions.

As previously mentioned a respiration rate of 30 to 60 bpm is typical of cattle under thermoneutral conditions, while 80 to 120 bpm are indicative of cattle under moderate thermal stress. When respiration rate exceeds 120 bpm cattle are considered to be under a high degree of stress (Mount, 1979; Hahn et al., 1997). Cattle with a respiration rate greater than 140 bpm are extremely stressed, and may die if action is not taken to reduce heat load. The respiration rate of heat-stressed cattle will fluctuate when heat load is maintained. At one point in time it may be 120 bpm, 10 minutes later it may fall to 80 bpm, and then later increases to 115 bpm. The reason for this has not been fully determined but it may be an attempt to maintain acid base balance in the blood as rapid breathing leads to reduced oxygen uptake.

Rapid shallow breathing or panting is energetically efficient and directs large volumes of air directly to tissues close to the core where metabolic heat is being generated. Unlike sweating, there is no loss of salts, and it allows for some cooling of blood supply to the brain (Silanikove, 2000). The evaporative cooling most likely occurs on the bronchial surfaces rather than bronchioles and deeper into the lung. High respiration rates can mean reduced tidal volume and over-ventilation may lead to blood pH changes due to excessive uptake of CO_2 from the pulmonary blood circulation (Bianca, 1965). Sustained panting over long but mildly hot conditions may cause respiratory alkalosis and a

concomitant rise in urinary pH from renal compensation (from decreased renal re-absorption of HCO_3^-).

Under high prolonged heat load, respiration rate may actually decrease as cattle shift from shallow rapid breathing to deep slower breathing. This represents a complete change in the respiratory dynamics of the animal and is an indication that the animal is failing to cope with the weather conditions. There is probably also a maximal breathing rate. A respiration rate ceiling for *Bos taurus*, *Bos indicus* and *Bos taurus x Bos indicus* cattle has been reported by Gaughan et al. (1999).

4.2.4 Appetite and eating behaviour

Reductions in feed intake due to high heat load are functions of the characteristics of the diet, diet type (e.g. grain or forage), the extent of insulation (hair), and metabolic activity (SCA, 1990). The effect of high heat-load on feed intake has mainly been largely measured in cattle confined to controlled climate facilities. Nevertheless, in grazing studies, Rittenhouse and Senft (1982) reported that the optimum ambient temperature for grazing activity changed from month to month. The shift was probably because of thermal acclimation by the cattle (Rittenhouse and Senft, 1982). Furthermore, the level of feed intake under hot conditions can be maintained at a level close to thermoneutral intakes by the provision of shade structures and by ensuring adequate night cooling (Muller and Botha, 1997; Holt et al., 1998; Sullivan et al., 2011). Ray and Roubicek (1971) reported that during the summer, most eating activity of feedlot steers occurred at sunrise and early evening. During winter most eating activity occurred in early afternoon. Field studies undertaken at a Queensland feedlot during summer demonstrated that cattle feeding activity closely followed natural feeding peaks at sunrise and sunset, with most feeding activity occurring at sunset (Lawrence, 1998).

An inverse relationship between ambient temperature and feed intake exists. During periods of high heat load dramatic drops in feed intake occur (Bianca, 1968; Gaughan et al., 2010b) (Figure 4.8). A 17% reduction in feed intake was reported by Brown-Brandl et al. (2005) for un-shaded heifers when mean ambient temperature increased from 19.7°C (maximum 21°C) to 27.7°C (maximum 35°C). A depression in intake of 3% to 5% has been reported to occur when ambient temperature increases from 25°C to 35°C; intake reductions in excess of 30% occur when temperatures exceed 35°C (NRC, 1981; Conrad, 1985; Mader, 2003). Dairy cows fed high energy rations (low acid detergent fibre (ADF) content) have greater reductions in DMI during hot weather than those fed rations with a higher ADF content (Staples, 2007), although the response is variable and dependent on the animal's thermal

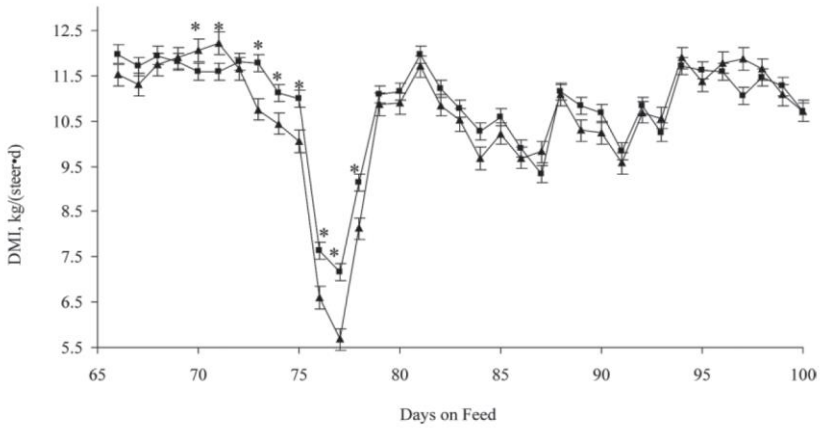

Figure 4.8. Reduction in dry matter intake when cattle are exposed to hot conditions (Gaughan et al., 2010b).

susceptibility, acclimation, and diet (Young, 1987). In a review on nutrition and heat stress in dairy cows it was reported that cows exposed to hot conditions results in lower ruminal fluid pH, less ruminating activity, lower milk fat percentage and reduced salivary buffering capability (Staples, 2007).

4.2.5 Water intake

The most critical nutrient is water. Water restriction will increase the negative aspects of high heat load by decreasing evaporative heat loss, which leads to further reductions in feed intake. Access to cool clean drinking water is paramount. Water intake may increase markedly during periods of high heat load e.g. in a feedlot study mean water intake increased from 32 L/steer/day to 82 L/steer/day as heat load increased (Gaughan et al., 2010b) (Figure 4.9). Therefore, feedlot managers need to ensure that there will be sufficient water (and access to water) to meet potential peak demands.

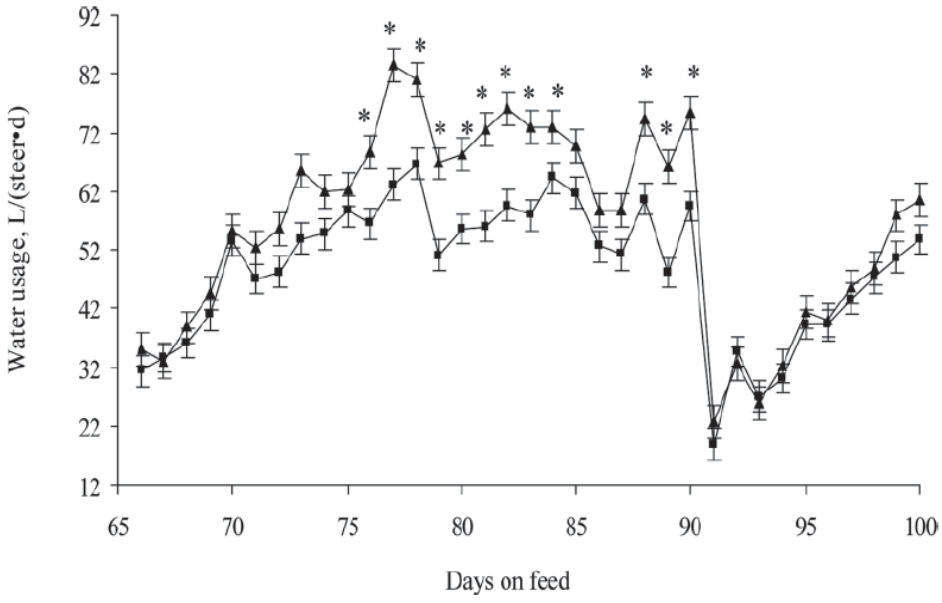

Figure 4.9. Increase in water consumption during a period of hot weather, followed by a large decline when hot conditions abated (Gaughan et al., 2010b).

Arias and Mader (2011) found water intake to be correlated to not only environmental conditions but feedlot surface conditions, as well. Water consumption in dairy cows is a function of dry matter intake, milk production and ambient temperature hence water requirements for lactating dairy cows are considerably higher even under mild conditions. For example, at 10°C a dairy cow producing 36 L of milk/day will consume 92 L of water/day, and at 27°C consumption increased over 30% to 132 L/day (Waldner and Looper, 2001). The effect of ambient temperature on water intake is less in low-producing cows; cows producing 14 L milk/d drank 78 L/d when ambient temperature was 28°C (Cowan et al., 1978). However as ambient heat load continues to increase there may be a decline in water intake. Studies have shown that the water intake of a 600 kg cow producing 27 kg milk/day was 120 L when held at an ambient temperature of 36°C but only 106 L when exposed to 40°C (NRC, 1981). This could be a function of accessibility or other behavioural challenges.

4.2.6 Breed/genotype differences

4.2.6.1 Genotype differences

The two main sub-species of cattle, the humped cattle of Indian origin (*Bos indicus* or zebu cattle) and the *Bos taurus* cattle breeds of Europe and Africa which for the most part do not have a hump, all arose from a common ancestor. However these two sub-species have undergone a separate evolution for several thousand years (Hansen, 2004). Much of the evolutionary difference is expressed in terms of stress tolerance, and in particular tolerance to heat stress (McDowell et al., 1953; Cartwright, 1955; Finch, 1986; Carvalho et al., 1995; Hammond et al., 1996; Gaughan et al., 1999). During their separate evolution from B*os taurus*, zebu cattle (*Bos indicus*) have acquired genes that confer thermo-tolerance at physical, physiological and cellular levels (Hanson, 2004). Figure 4.10 illustrates that cattle from zebu breeds are better able to regulate rectal temperature and sweating rates in response to heat stress than cattle from a variety of *Bos taurus* breeds.

It is generally accepted that *Bos indicus* genotypes have greater heat tolerance than *Bos taurus* genotypes. There are however exceptions, for example, the Tuli, closely related to *Bos taurus* but tropically evolved, appears to have a high degree of heat tolerance (Hammond et al., 1998). A Queensland, study found that when given access to shade during periods of hot weather, Brahmans sought shade the least and Shorthorn's the most (Bennett et al., 1985). The Shorthorn steers spent 1 hour longer in the shade each day than did the Brahman steers. The Brahman also had lower rectal temperature and respiration rate than the Shorthorn cattle. However, others have reported that the rectal temperature of Brahman cattle and Angus cattle (40.0 and 40.9°C respectively) were higher than the rectal temperature of Senepol cattle (39.6°C) under the same conditions (Hammond et al., 1998).

The effects of heat load on different breeds of cattle (*Bos indicus*, *Bos taurus* and *Bos indicus* × *Bos taurus*) have been reviewed by a number of authors (Bianca, 1965; Finch, 1986; Hammond et al., 1996; Morrow-Tesch and Hahn, 1994; Hammond et al., 1998; Gaughan et al., 1999). *Bos indicus* breeds (e.g. Brahman) although having greater heat tolerance than *Bos taurus* breeds, often have lower productivity (growth rate and reproductive efficiency) than the less heat-tolerant breeds (Gaughan et al., 2009a).

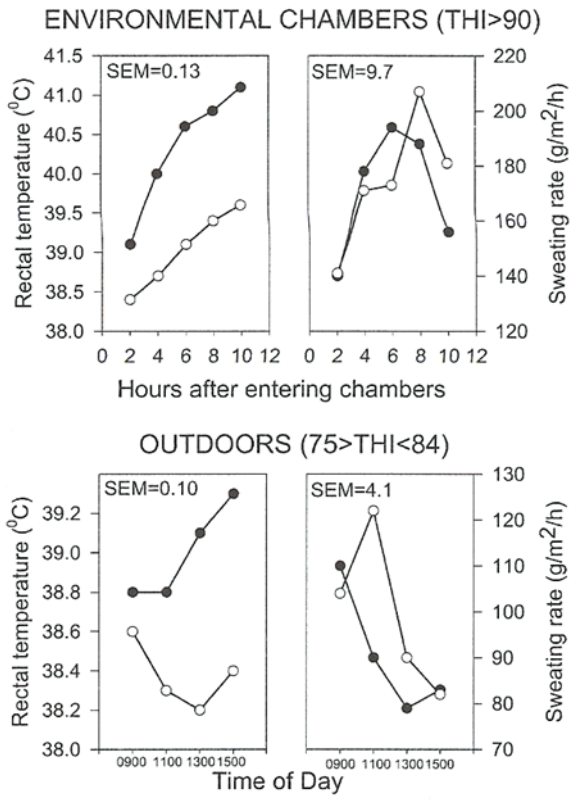

Figure 4.10. Differences between Hereford (closed circles) and Brahman (open circles) in regulation of rectal temperature and sweating rate (Hansen, 2004).

Chowdhury and Sadhu (1961) reported that the structures common to Indian Zebu cattle, such as the hump, voluminous dewlap and navel flap are specially developed in regions with oppressive summer heat, and suggest that these special structures may be related to the animal's greater thermoregulatory ability. However, a study by McDowell et al. (1958) assessed the relationship of the *rhomboideus* (hump) muscle in Zebu and European type cattle, and found that the surgical removal of the dewlap and hump of Red Sindhi bulls did not have a significant effect on thermoregulation.

The relationship between rectal temperature and sweating rate was investigated by Finch et al. (1982). They reported a stronger link between sweating rate and rectal temperature for Brahman cattle (B) than for the Brahman × Shorthorn (BX) or Shorthorn cattle (S). Early studies reported that the density of sweat glands in *Bos indicus* was greater than for *Bos taurus* cattle (Ferguson and Dowling, 1955), and that *Bos indicus* cattle had larger and more numerous sweat glands (Nay and Hayman, 1956). This may explain why the range and mean rectal temperature in Brahman cattle were lower and only slightly affected by environmental heat (Figure 4.11).

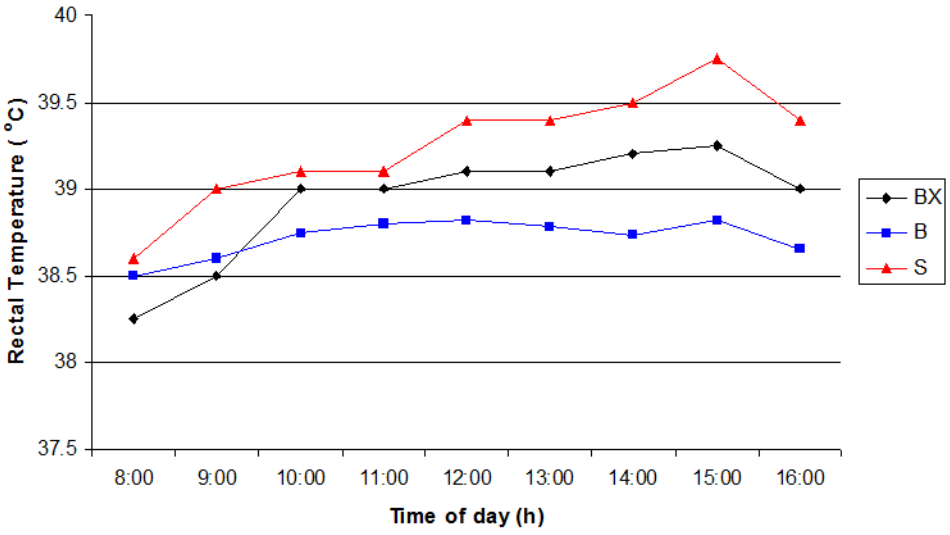

Figure 4.11. Mean rectal temperatures for each hour of measurement for each breed (adapted from Finch et al., 1982)

Breed differences in the rate of evaporative cooling from the respiratory tract have been reported. *Bos taurus* cattle reach maximal vaporization rates at approximately 27°C, while *Bos indicus* cattle reached peak evaporation rates at 35°C (Yousef et al., 1968). The respiration rate (as opposed to the mean respiration rate over 24 hours which may be similar) is considerably lower for *Bos indicus* cattle than for *Bos taurus* cattle, and this is thought to be an indicator of the heat tolerance of *Bos indicus* cattle. A study by Rhoad (1936) examined differences in the respiration rate of Zebu, Holstein and Zebu × Holstein cattle when exposed to hot conditions (35˚C). He reported that the respiration rate of Zebu cattle was approximately 30 bpm while the Holsteins had a respiration rate of over 100 bpm. Whereas the respiration rate of Holstein × Zebu crosses was somewhat intermediate at approximately 80 bpm. Olbrich et al. (1973) reported a respiration rate of 122 bpm for *Bos taurus* heifers and 25 breaths/minute for *Bos indicus* heifers at 31°C. Hammond et al. (1998) found that the respiration rate of Brahman and Angus heifers was 39 bpm and 74 bpm respectively at a THI[1] of 84 units. During the same study the respiration rate of Senepol and Tuli × Angus heifers was 50 and 60 bpm respectively.

In a study conducted by Gaughan et al. (1999) five genotypes were assessed for heat tolerance. The genotypes used were: Hereford (H), Brahman (B), H × B, H × Boran (H × Bo), and H × Tuli (H × T). The Tuli is a tropically adapted *Bos taurus* breed from southern Africa, and the Boran is a *Bos indicus* breed also from southern Africa. Under extremely hot conditions (mean temperature humidity index (THI > 90), Brahman had lower ($P<0.05$) rectal temperatures and respiration rates than the other genotypes (Table 4.2), which may be indicative of greater surface area per mass to dissipate heat and lower metabolic rate than other genotypes (Gaughan et al., 1999).

[1] THI = temperature humidity index ($THI = (0.8 \times T_A) + [(RH \times 0.01) \times (T_A - 14.4)] + 46.4$), where T_A is dry bulb temperature (°C) and RH is relative humidity (%). However there are some variations to this formula in the literature (see Gaughan et al., 2012)..

Table 4.2. Rectal temperature (RT) and respiration rate (RR) of Hereford (H), Brahman (B), H × B, H × Boran (H × Bo), and H × Tuli (H × T) genotypes during a 10 hour heat stress period (mean THI > 90).

	Genotype					
	H	B	H x B	H x Bo	H x T	SE
RT, °C	40.3	39.0	40.0	39.5	39.5	0.06
RR, bpm[1]	168	104	139	171	166	4.4

[1] bpm = breaths per minute; (adapted from Gaughan et al., 1999).

Three Zebu and three Highland mature heifers were used in a study by Seif et al. (1979) to determine the effect of a moderately high environmental temperature (31°C) on temperature-regulatory responses of cold- and heat- tolerant cattle to thermal stress. Following an adjustment period to 31°C, the Highland heifers decreased their internal heat production by decreasing feed consumption by 31% and oxygen consumption by 19%. Water consumption increased by 190%. The Zebu decreased feed consumption by 19%, increased respiration rate 100% and water consumption by 50%. Johnston et al. (1958) concluded that the greater heat tolerance of Indian type cattle is due to lower basal heat production rather than a more efficient heat loss mechanism.

The physiological responses of *Bos taurus* and *Bos indicus* cattle to prolonged, continuous heat and humidity were studied by Beatty et al. (2006). In these studies a linear increase of wet bulb temperature and respiration rate was observed in both genotypes as the wet bulb temperature rose from 26 to 32°C. The prolonged exposure to heat and humidity caused an increase in core body temperature for both genotypes indicating that the animals heat loss mechanisms could not compensate fully for the excessive heat load. Associated with the rise in core body temperature were clinical signs of heat stress in the *Bos taurus* animals including open-mouthed panting, drooling, reluctance to stand/rise, increased licking of coat, and general dullness including neurological signs of staring and glazed eyes. No clinical signs of heat stress were reported for the *Bos indicus* cattle (Beatty et al., 2006). The *Bos indicus* in this experiment had an increase of core body temperature of 2.3°C from the lowest recorded mean core body temperature.

4.2.6.2 Phenotypic differences

The reflective properties of skin and hair coat of cattle, and the physical properties (e.g. density, length) of hair are important factors in heat transfer between the animal and the environment.

Coat colour

Early studies undertaken in South Africa by Riemerschmid and Elder (1945) (cited by Findlay, 1950) showed the importance of coat colour on absorption of solar radiation (Table 4.3). A later study found that cattle with a black-coat had an absorbance (α) of 1 from direct radiation; a white-coated cattle has an absorptance of 0.37 and a red-coated cattle an absorptance of 0.65 (Cena and Monteith, 1975). Slightly lower values of 0.92 where reported for Angus and Holsteins by da Silva et al. (2003). The reflectance (ρ) of different coat colours in beef cattle was reported by da Silva et al. (2003). They reported that the reflectance from black coated cattle (Brangus) was 0.05, for dark grey Nelore ρ = 0.28, for light grey Nelore ρ = 0.56, for Red Simmental ρ = 0.58, for white Simmental ρ =

0.67, and a slightly lower value for another white breed (Canchim) ρ = 0.60. The differences between the white breeds may be due to hair characteristics (da Silva et al., 2003). From this we can deduce that lower reflectance results in a potential increase in body temperature.

Table 4.3. The mean absorptivity to solar radiation for hides of different colours.

	White[1]	Cream	Red[1]	Dark Red	Black
Mean Absorptivity (%)	49	50	78	83	89

[1] *Bos indicus* breeds (Adapted from Findlay, 1950)

Hillman et al. (2001) reported that when black cows were exposed to sunlight, their surface temperature (not skin temperature) increased by 4.8°C whereas white cows had an increase of 0.7°C. Considerable differences in the amount of sensible heat gain between dark-red hair coats, black, tan and white beef heifers standing in direct sunlight was reported by Hillman et al. (2005). In that study the dark-red heifers had a 26% increase in sensible heat flux compared with black at 22%, tan at 5% and white at 4%.

Heat stress related mortalities of feedlot cattle in the USA (1995 and 1999), and in Australia (1991 and 2000) occurred in pens which held both dark coated and light coated cattle. Investigations into cattle deaths from US heat waves found that in some feedlot pens 80% of the deaths came from the 20% of the cattle which were dark-coated (Busby and Loy, 1996; Mader pers. comm. 2010). Similar findings were reported by Entwistle et al. (2002) who investigated cattle deaths during the Australian heat wave of 2000. These data suggest that the dark coated cattle are more susceptible to the effects of high heat load. However, Walsberg (1983) stated that "there is no simple relation, even in a qualitative sense, between coat colour and radiative heat gain". Indeed, studies set up to determine the effect of cattle coat colour on heat tolerance have produced contradictory results. Some studies have shown that cattle with darker coat colour had lower body temperatures and better growth performance than light coat coloured animals (Schleger, 1962; Finch and Western, 1977; Peters et al., 1982). Other and more recent studies have shown that cattle with dark coat colour acquire greater solar heat load (Bonsma, 1949; Finch et al., 1984; Gaughan et al., 1998), have lower performance and are more likely to seek shade in hot environments (Gaughan et al., 1998; Sullivan et al., 2011). Also, two studies reported that dark-hide cattle had higher respiration rates, panting scores and skin temperatures than light coloured cattle(Brown-Brandl et al., 2003; 2006).

Coat type

The hair coat of cattle can be characterized largely by fibre density, hair length and optical properties. Furthermore, the characteristics of the hair coat of cattle are influenced by genotype and phenotype. It is generally accepted that *Bos taurus* type cattle typically possess a deep woolly coat whereas the *Bos indicus* type cattle have a coat which is made up of sleek, dense and thin hair (Yeates, 1977; Finch, 1986). The deep woolly coat of the *Bos taurus* impedes both evaporative and non-evaporative heat loss (Findlay, 1950; Yeates, 1977). This is beneficial during periods of cold weather but is detrimental when *Bos taurus* cattle are exposed to high heat load. A woolly coat reduces conductive and convective heat flow, and evaporative heat loss, which under hot condition may exacerbate the effects of heat stress (Berry et al., 1962; Finch et al., 1984). In still air heat

exchange through the coat or boundary layer will be primarily by movement of heat through entrapped air and along hair fibres (Gebremedhin, 1985).

When air temperature exceeds skin temperature, or if an animal is in the sunlight, the movement of heat through the coat will result in a net inward flow of heat through the coat or skin. For this reason, resistance of the animal coat to environmental heat flow is important for body temperature control (Finch, 1986). To maintain the same control over core body temperature as *Bos indicus* cattle, *Bos taurus* cattle need to lose approximately 20% more heat from the skin via evaporation (Finch, 1986) than *Bos indicus* cattle.

The dense flat coat of the *Bos indicus* type cattle provides greater resistance to heat transfer to the skin, due to the smooth surface reflecting radiation at or near the surface when the animal is exposed to solar radiation (Finch, 1986; Hansen, 2004). When the coat hair of Shorthorn cattle (*Bos taurus*) was clipped there was a reduction in the magnitude of hyperthermia in response to heat stress (O'Bannon et al., 1955). There is a correlation between body temperature and productivity of beef cattle in the tropics, where sleek dense coats are associated with lower body temperatures and higher growth rates when compared to deep woolly coated cattle (Findlay, 1950; Turner and Schleger, 1960; Peters et al., 1982). This has been recently demonstrated in dairy cattle by Dikmen et al., (2008) who was able to show that slick-haired Holstein cows were able to regulate body temperature more effectively (< 39°C) than wild-type Holstein cows (> 39°C) when exposed to an acute increase in heat stress (THI ranging from 81.4 to 84.4). The increased thermal resistance of the slick-haired animals was likely due to the increased evaporative cooling and reflective radiation properties.

4.3 Endocrine responses

Any stressor will redirect endocrine and metabolic processes toward maintenance of homeostasis and away from growth. Reduced feed intake in response to increased thermal conditions is observed in most mammalian species, but the endocrine regulation and molecular machinery behind this response is poorly described (Matteri et al., 2000). Acclimation to thermal stress is understood to be a homeorhetic process (Collier et al., 2006a). The concept was first described by Bauman and Currie (1980) and it refers to “the coordinated changes in metabolism of body tissues necessary to support a physiological state”. Homeorhetic control is proposed to involve multiple tissues and pathways and occur over days if not weeks. The paracrine and endocrine signals altering metabolic and behavioural responses work on a system wide basis and down to organelles within cells of many different tissues. The workings of these processes in heat stress have enlisted little research despite the extent of the problem (Collier et al., 2008). Most investigations of ruminant endocrine responses in heat stress have been conducted in lactating dairy cows.

Collier et al. (2006a) suggested that there is evidence for a biphasic pattern of heat acclimation; acute and chronic phases inducing acute and acclimation changes which necessarily invoke different endocrine and subsequent metabolic responses. The acute phase consists of changes in cellular signalling pathways leading to cellular reprogramming to minimise the immediate negative effects of heat stress. The chronic phase is proposed to take over at the end of the acute phase, with expression of heat acclimated phenotype. Genetic differences in heat tolerance also become obvious (Collier et al., 2006a). Adaptations to chronic heat stress include decreased basal

metabolism and increased water and electrolyte metabolism along with the concomitant endocrine and cellular changes (Beede and Collier, 1986).

4.3.1 Stress hormones

The acute and acclimation heat stress responses are initiated by the hypothalamic-pituitary-adrenal axis and results in altered secretion and circulating levels of several hormones including corticotropin-releasing hormone, adrenocorticotropic hormone (corticotrophin), cortisol and aldosterone (Collier et al., 2006a).

Cortisol is the main glucocorticoid in cattle. It has gluconeogenic and lipolytic effects on the liver by driving hepatic conversion of FFAs and amino acids into gluconeogenic substrates (Matteri et al., 2000). As in other acute stress stimuli, an acute heat stress episode in cattle will induce a rapid increase in plasma cortisol levels. Cortisol levels will rise within 20 minutes of exposure to acute heat stress and achieve a plateau after 2 hours (Silanikove, 2000). Abilay et al. (1975) noted plasma cortisol increased in Holstein steers over the first hour of a 4 hour exposure to 42°C and 60% RH. A plateau was achieved at 160 minutes with three-fold increase above normal cortisol levels. This was followed by a small fall in levels at 200 minutes. On rapid cooling, plasma cortisol quickly returned to basal levels. Alvarez and Johnson (1973) working with non-lactating Holsteins also observed a rapid rise in serum glucocorticoids on exposure to 40°C, but the increased levels did not persist, falling to below normal levels after 4½ hours exposure. Similarly, exposure of non-lactating Holsteins to 3 days at 35°C also induced a transient increase in glucocorticoid levels, peaking at 2 hours, and then declining after 4 hours exposure (Alvarez and Johnson, 1973).

While the glucocorticoids increase in acute heat stress, the increased levels are not sustained in chronic heat stress or during the acclimation phase (Beede and Collier, 1986). In prolonged hot conditions, cortisol levels fall, but a re-emergent increase may indicate the onset of real distress. Hammond et al. (1996) argued that cortisol has no role in the development of heat tolerance. In dairy cattle, glucocorticoid levels decreased during acclimation at 35°C and were lower in thermally acclimated animals compared with controls (Collier et al., 2006a). With 24 days exposure to 35°C, the glucocorticoid levels in non-lactating Holsteins was 40% higher than thermoneutral controls at day 3, and were observed to decline through the remaining period (Alvarez and Johnson, 1973). Interestingly, Zebus have a higher basal plasma cortisol levels (Hammond et al., 1996) and may not be as responsive to acute heat stress. Zebu heifers did not experience a rise in plasma cortisol till 140 minutes of heat exposure (Abilay et al., 1973).

The catecholamines, adrenalin (epinephrine) and noradrenalin (norepinephrine) are also gluconeogenic and lipolytic hormones (Matteri et al., 2000). Adrenaline and noradrenaline increase in both acute and chronic heat stress. These hormones may have a role in promoting sweat gland activity since cattle sweat glands are not directly innervated, but are under adrenergic controls (Beede and Collier, 1986). Alvarez and Johnson (1973) monitored adrenalin and noradrenalin levels in their heat stress experiments on non-lactating Holsteins, and found sustained high levels of the catecholamines with prolonged exposure to high temperature. Acute heat stress drove serum adrenalin and noradrenalin levels two-fold higher than controls after 4½ hours exposure at 40°C, whereas more moderate conditions (35°C for 3 days) saw adrenalin and noradrenalin levels peak at 8 hours, with a 67% increase over normal levels, which persisted for the remaining 2 days. With 24

days exposure to 35°C, the high adrenalin and noradrenalin levels continued to rise to 100-180% higher than controls. While the adrenalin levels remained higher than normal throughout, noradrenalin levels fell to 40% higher than normal after day 18. Since the adrenal medulla is unlikely to sustain high levels of circuiting catecholamines, it is most probable that the sympathetic nervous system is the main contributor in chronic heat stress. Aldosterone, which is secreted by the adrenal cortex and imposes Na^+ conservation, decreases in heat-stressed ruminants (Beede and Collier, 1986). Ruminants produce sweat with high K^+ (unlike non-ruminants) thus heat-stressed ruminants need to conserve K^+ rather than Na^+.

4.3.2 Metabolic hormones

The hypothalamic hormone, corticotropin-releasing hormone, prompts the release of hypothalamic somatostatin, which in turn influences secretion of growth hormone (GH, somatotropin) and thyroid stimulating hormone from the pituitary (Collier et al., 2006a; Matteri et al., 2000).

The activities of the thyroid hormones account for 50% of basal metabolic rate; thus they have strong influence over thermogenesis. A 0.6°C increase in rectal temperature leads to a marked reduction in thyroid activity (Bianca, 1965). Plasma T_3 and T_4 concentrations decrease by ~25% in heat stress over several days to obtain a new steady state level (Silanikove, 2000). The reduced secretion of T_3 and T_4 results in decreased basal metabolic rate and thus reduced heat production (Bernabucci et al., 2010). The magnitude of thyroid response depends of the original level of activity and is affected by genetics. Thyroid activity of Herefords is higher than that of Brahman, and higher in dairy cattle than beef cattle. Heat-stressed young heifers also experience decreased T_3 and possibly increased T_4 (Nonaka et al., 2008).

GH (somatotropin) enables access to energy reserves by inducing mobilisation of NEFA from adipose, increasing hepatic gluconeogenesis, and reducing glucose uptake by muscle. Increased GH levels are thought to counteract the action of insulin, thus preserving glycogen and other gluconeogenic stores (Matteri et al., 2000). It is also responsible for about 80% of hepatic insulin-like growth factor-1 (IGF-1) production. IGF-1 is a hepatic protein hormone synthesized in response to the pituitary derived growth hormone and hypothalamic derived somatostatin. It drives the growth and development of numerous tissues. Generally circulating GH (and correspondingly, IGF-1) levels fall in response to stress in most species. However, a simultaneous increase in plasma GH and decrease in circulating IGF-1 has been observed in stress and this response is thought to act as a brake on growth and to divert energy to survival (Matteri et al., 2000).

Reports on altered plasma GH levels in cattle in response to heat stress are inconsistent (Bernabucci et al., 2010). The GH response is likely to be highly dependent on the phase of the heat stress response: acute or acclimatory, as well as the age and gender of the animals. Mitra et al. (1972) observed decreased plasma GH and GH secretion rate and a decrease in the overall GH pool in heat acclimatized 4-5 year old nonlactating Jersey cows. McGuire et al. (1991) recorded a tendency (but not significant) to lower plasma GH in lactating cows exposed to 12 hours at 40°C and 70% RH for 8 days. In this experiment which also assessed the effects of feed restriction, there was no change in IGF-1 plasma levels. Heifers on a finishing diet over normal summer conditions recorded consistently lower plasma IGF-1 relative to winter measurements (Mader and Kreikemeier, 2006).

The endocrine and metabolic response to the reduced feed intake that is concomitant with heat stress has confounded attempts to understand the consequences of heat stress. Rhoads and colleagues (University of Arizona) have established an excellent experimental model that discriminates the impacts of reduced feed intake from the effects of heat stress in dairy cows. In parallel with the heat stress treatment group, the control group of cows are pair fed the same DMI as the heat-stressed group while housed in thermoneutral conditions. In this design, Rhoads and colleagues have consistently measured a slightly decreased plasma IGF-1 in heat-stressed cows relative to pair-fed thermoneutral controls (Rhoads et al., 2009a, b; 2010). This was supported by evidence of reduced hepatic transcription of the IGF-1 gene (Rhoads et al., 2010). However, this reduced expression was not due to reduced plasma GH, despite decreased plasma somatostatin which normally suppresses GH production (Rhoads et al., 2009a). Studies of heat-stressed in cattle also found decreases in both plasma somatostatin and IGF-1 levels (Bernabucci et al., 2010). Chronically but mildly heat-stressed sheep (24 days cycling between 17-36°C) whose feed intake was not affected also tended to be lower plasma IGF-1 (Dunshea et al., 2012; unpublished).

In looking more closely at the hepatic response in heat stress (in lactating dairy cows), GH receptor (GHRc) expression was apparently reduced as well as the phosphorylation of STAT5 (the signal transducer employed by GHRc). In contrast, GHRc transcript expression in pair-fed animals is unchanged or possibly increased (Rhoads et al., 2010). This group of researchers suggested that heat stress favours uncoupling of the relationship between plasma GH and hepatic IGF-1 synthesis. However, when recombinant bovine somatotropin (rbST, the recombinant version of GH) was administered to heat-stressed cows and their pair-fed counterparts, both groups responded with a three- fold and two-fold increase IGF-1 transcript expression in the liver, respectively, demonstrating that the hepatic IGF-1 response to GH is not impaired although sensitivity may have been attenuated (Rhoads et al., 2011).

Heat stress will induce increased insulin production and responsiveness, despite the reduced feed intake. In normothermic conditions, underfeeding usually drives down insulin production and secretion. The increased insulin level possibly counteracts the actions of lipolytic stress hormones (Bernabucci et al., 2010). The increased insulin in heat stress is consistent across species, age and lactation status, although basal plasma insulin levels are lower in *Bos taurus* than *Bos indicus* (Beatty et al., 2004). For example, 6-month old Holstein bull calves in heat stress underwent a 30% increase in plasma insulin compared to pair-fed counterparts (O'Brien et al., 2010). Similarly, increased plasma concentrations of insulin and heightened insulin responsiveness were observed in heat-stressed lactating dairy cows (Wheelock et al., 2010). Baumgard (2011) succinctly differentiated the underfed animal as maintaining 'metabolic flexibility' by regulating insulin sensitivity as compared to the 'metabolically inflexible' heat-stressed animal which has not or cannot adjust insulin sensitivity.

Prolactin is another homeorhetic hormone that has received some attention in relation to heat stress. Alongside its acknowledged role in reproduction and lactogenesis, prolactin is implicated in salt-water balance, growth and development, and metabolism (Matteri et al., 2000). It is also associated with passive coping responses. Plasma prolactin levels respond to increased ambient temperature and photoperiod (Collier et al., 2006a, Bernabucci et al., 2010). Plasma prolactin levels increase in acute heat stress in cattle despite decreased feed intake (Beede and Collier, 1986). However, plasma prolactin was seen to decrease when dietary intake of K^+ was increased in heat

stress; thus prolactin may have a role in K^+/Na^+ conservation and regulation (Beede and Collier, 1986).

Hypothalamic and gut peptide hormones that participate in appetite controls are most probably involved in heat stress responses. Thus far, very little is known of the involvement of the neuropeptide Y (NPY) family and ghrelin in heat stress in any species (Matteri et al., 2000) but their likely participation is supported by a recent report of increased gene expression of ghrelin in the hypothalamus and gastrointestinal tract of chronically heat-stressed chickens (Song et al., 2012). In contrast, cholecystokinin gene expression was reduced in these tissues.

In summary, under heat stress, the endocrine status of ruminants, generalised from our understanding of responses in dairy cows, is one of an early but unsustained cortisol rise, and high and continuous secretion of adrenalin and noradrenalin. Aldosterone is reduced to conserve K^+. Metabolic rate is lowered through reduced secretion and actions of the thyroid hormones, T_3 and T_4, and reduced somatostatin and IGF-1. The GH response is less consistent maybe specific to animal and environmental factors. Insulin production and sensitivity is increased and this has significant implications for energy metabolism.

4.4 Metabolic responses

The majority of studies on metabolism in heat stress have been conducted in dairy cows with early work looking at simple plasma indicators in beef cattle conducted in the 1970's. Over the decades, definite changes in response to heat stress has been described in energy metabolism, nitrogen uptake and utilisation, rumen function, electrolyte balance and blood buffering and oxidative status. As with much of the work reviewed here, the studies are variously diverse looking at different stages of lactation, gender, age, and different breeds all presented with different thermal environmental challenges. As alluded to above, a major confounding factor is the underlying reduction of feed intake which under thermoneutral conditions elicits a well understood metabolic response. The definitive "story" of what is different about voluntary feed restriction during heat stress has not yet evolved however, some recent studies in energy metabolism is starting to give answers.

4.4.1 Carbohydrate and fatty acid metabolism

Using their pair-fed thermoneutral vs. heat-stressed experimental design, Rhoads and colleagues have consistently found that reduced feed intake accounts for about 50% of heat stress induced reduction of milk yield which also causes a 50% reduction in lactose produced by heat-stressed cows (Baumgard et al., 2011; Wheelock et al., 2010). These very overt changes reflect the altered metabolic profile of heat-stressed cows compared to thermoneutral control cows on reduced feed intake. Generally, heat-stressed cows are hypoglycaemic with a 5-10% decrease in blood glucose; most likely a consequence of increased basal and glucose stimulated plasma insulin (Wheelock et al., 2010). Furthermore, glucose disposal rates are increased alongside lower glucose appearance rates relative to pair-fed counterparts (Baumgard et al., 2011; Wheelock et al., 2010). Despite displaying a similar glucose response when subjected to an adrenaline challenge, heat-stressed cows under glucose tolerance testing presented a higher glucose clearance rate than pair-fed counterparts, indicating that heat stress triggers a 'conventional' stress response rather than a change in sensitivity of the glucose-IGF-I axis to adrenaline.

Rhoads et al. (2011) assessed liver function by measuringthe hepatic gene expression of two enzymes integral to the gluconeogenic pathway, pyruvate carboxylase and phosphoenolpyruvate carboxykinase-1(PEPCK). PEPCK is the rate controlling enzyme in gluconeogenesis, and its expression is precisely regulated by cortisol and pancreatic glucagon. Pyruvate carboxylase provides PEPCK with its substrate, oxaloacetate. Expression of pyruvate carboxylase is also upregulated by cortisol and pancreatic glucagon, but down regulated by insulin. Pyruvate carboxylase transcript expression was increased by 50% in both heat stress and pair feeding treatments. PEPCK transcript abundance differentiated the two treatments: PEPCK expression was increased in the pair-fed group but not in the heat-stressed group. Thus gluconeogenesis appears constrained by lack of response of the PEPCK gene.

Increased levels of circulating NEFA are expected in animals undergoing reduced food intake. Interestingly, heat-stressed cows, which voluntarily reduce their feed intake, do not seem to mobilize NEFA (Wheelock et al., 2010; Baumgard et al., 2011). Even under adrenaline challenge, the heat-stressed cows had 50% lower NEFA response than pair-fed animals (Baumgard et al., 2011). With the apparent deficiencies in NEFA mobilisation, Wheelock et al. (2010) have argued that glucose is the favoured fuel in heat stress, with increased insulin driving increased glucose consumption, and suggest that the suppression of lipid mobilisation in the heat-stressed animals is an adaptive response since cold-stressed animals have both increased glucose and NEFA. Baumgard et al. (2007) postulated that the reason for this metabolic adaptation was that a higher heat cost was associated with accessing ATP from NEFAs relative to glucose.

Of relevance to this review is whether this apparent perturbation in energy metabolism pertains to non-lactating animals. Beatty et al. (2004) found that Angus heifers heat-stressed for 5 days mobilised NEFA as effectively as their pair-fed counterparts, and triglyceride levels were unaffected by heat stress. Additionally, there was no difference in plasma glucose or lactate between the heat-stressed and pair-fed groups. On the other hand, Nonaka et al. (2008) working with prepubertal Holstein heifers subjected to 14 days at 33°C found a tendency to increase energy retention as fat by 2.3-2.5 MJ/day and decrease retention of energy as protein. These heat-stressed young heifers also recorded lower glucose, NEFA, triglyceride and cholesterol levels (Nonaka et al., 2008).

Working in young Holstein bull calves, O'Brien et al. (2010) reported decreased plasma glucose (relative to pair-fed controls) which was likely to be driven by the 30% increased plasma insulin. The glucose response in the heat-stressed calves was blunted and relative to pair-fed controls, the heat-stressed calves had reduced plasma NEFA. Exposure of steers to heat stress induced decreased plasma cholesterol, phospholipids, lysolecithin and sphingomyelin with a decrease in the unsaturated fatty acids associated with these fractions (O'Kelly, 1973; O'Kelly and Reich, 1975). The Brahman cross steers also recorded reduced plasma NEFA and reduced plasma glucose induced by heat stress (O'Kelly, 1973). The indicators from these reports suggest that growing cattle may adapt their energy metabolism in a similar manner as outlined for dairy cattle (see Figure 4.12). This scenario for beef cattle is yet to be confirmed and requires development of a parallel experimental design along the lines developed by Baumgard, Rhoads and colleagues.

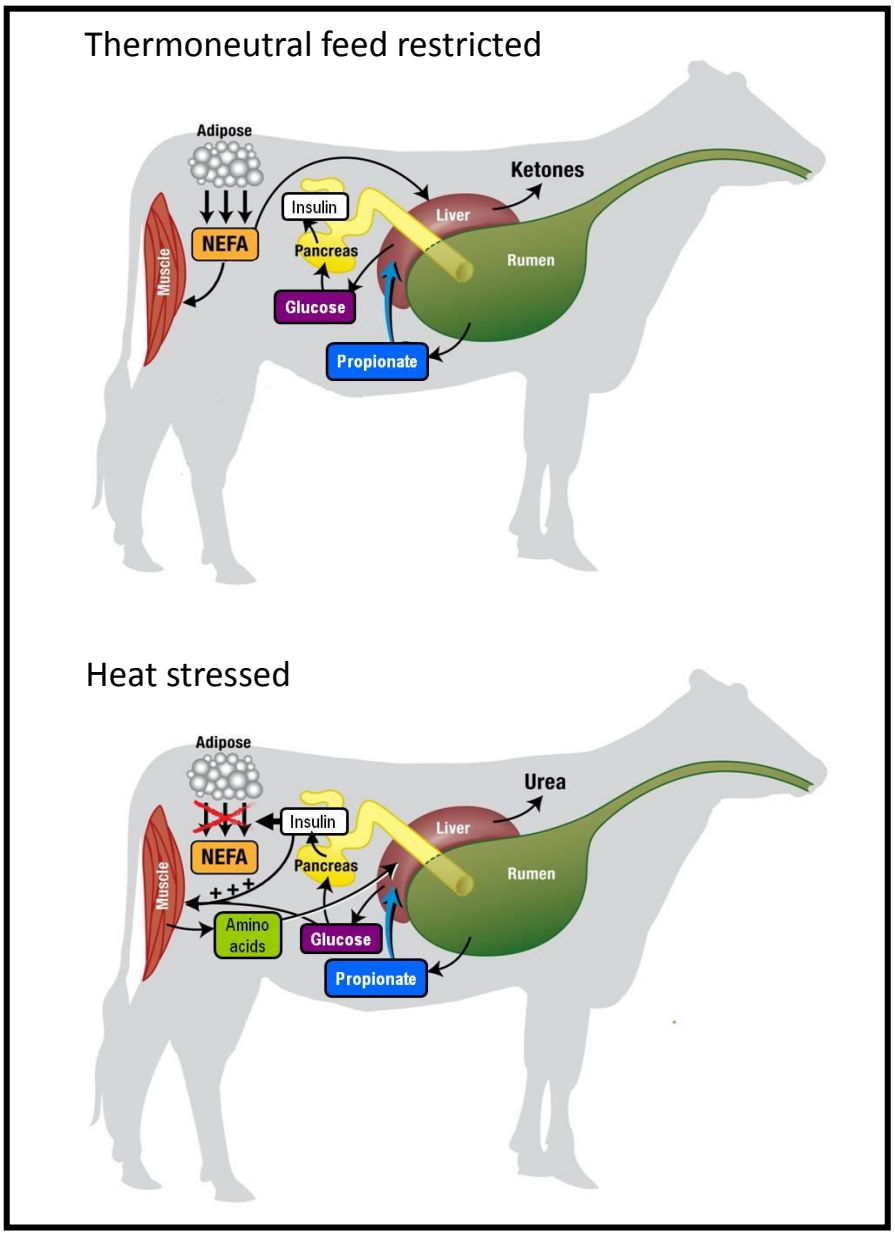

Figure 4.12. A schematic describing the hypothesized altered energy metabolism of heat-stressed steers, and extrapolated from studies on lactating dairy cows (adapted from Baumgard, 2011). In heat stress, glucose is the preferred energy metabolite, with NEFA in adipose not being accessed, and amino acid from muscle is mobilised to be consumed by the liver as the main glucose producing metabolite.

4.4.2 Nitrogen metabolism

Increased plasma urea nitrogen (PUN) along with increased excretion of urea N in the urine are acknowledged consequences of heat stress, but the contribution of reduced feed intake vs. heat stress alone to N metabolism is still not understood. Hassan and Roussel (1975) found a positive correlation between rectal temperature and blood non-protein N in lactating Holsteins subjected to thermal stress conditions for three weeks. The increased plasma urea levels may be due to the combination of decreased ammonia uptake by gut microbiota and increased hepatic deamination of

amino acids from muscle (despite the insulin stimulus for increased protein synthesis in muscle) or release of amino acids from peripheral tissues especially muscle.

Hepatic urea N can either be excreted via the kidneys as urinary urea N or enters the gastrointestinal tract from the blood via exocrine secretion through the gut epithelium. Most hepatic urea N is recycled between the gut and tissues, thus increased urea N excretion in heat stress indicates an effect on urea N recycling. Urinary urea N levels reflect ammonia detoxification and amino acid (protein) catabolism. The endogenous urea N in the gut undergoes hydrolysis by gut microbial ureases and then may be excreted into faeces as N compounds, re-enter the hepatic orthinine cycle, or be converted to amino acids in the rumen and tissues. This last anabolic process indicates the efficiency of N recycling.

Heat-stressed dairy cows with significantly lower DMI will necessarily have reduced N intake; however, urinary urea N excretion is frequently increased. In mildly heat-stressed cows, Kamiya et al. (2006) found increased urinary urea N excretion but no increase in urinary 3-methyl histidine (3MH). This modified amino acid produced by the methylation of histidine in actin and myosin, is often used as a marker of myofibrillar degradation. Plasma 3MH was increased (two-fold) as was PUN and milk N urea. By comparison, cows on 70% DMI had lower urinary urea N excretion, lower plasma 3MH, and plasma urea N and milk urea N were not affected. In heat-stressed young Holstein heifers, PUN increased significantly and 3MH tended to increase (Nonaka et al., 2008). Similarly, O'Brien et al. (2010) noted significantly increased PUN (75%) but for only days 2-4 of a 9 day treatment of young Holstein bulls.

Obitsu et al. (2011) compared mildly heat-stressed cows to those at cool ambient temperature, thus the effects of reduced feed intake were unaccounted for. As may be expected, N intake, faecal N excretion, digestible N, milk N and retained N/day were all reduced significantly. Total urinary N was not affected but the ratio of urinary N: N intake was significantly increased (despite the reduced DMI), thus actual urea N production increased. The sources for increased N production are release of amino acids from tissues such as muscle, and or increased absorption of N compounds from the gut. PUN, plasma creatinine, urinary urea N and creatinine excretion were significantly increased also pointing to increased muscle degradation.

McGuire et al. (1989) went on to look at plasma portal flow since this parameter and the net flux of metabolites from the gut to the circulatory system are affected by feed intake, and found a 14% reduction in plasma portal flow in both feed restricted and heat-stressed cows. Net flow of amino N was reduced by 20% and 35% respectively. Heat-stressed cows also experienced decreased net absorption of α amino N relative to the feed restricted cows. McGuire et al. (1989) suggested that heat-stressed induced redistribution of blood flow to the rumen and intestine may explain much of the altered N flux. Thus the profile of a mildly heat-stressed ruminant is one of decreased portal flow with reduced amino N uptake but increased NH_3 from reduced incorporation in microbiota protein (see Figure 4.13). The liver itself is producing more NH_3 from increased deamination of amino acids released from tissue and muscle protein. The accumulating amounts of NH_3 is captured as urea N production by the liver, and discharged in the blood stream to be mostly expelled by the kidney into urine. How and to what extent these adaptations are different to a feed restricted ruminant in thermoneutral conditions awaits elucidation.

4.4.3 Rumen fermentation

The fermentation process in the rumen is also affected by heat stress, and at an ambient temperature of 40°C, feeding and rumination usually ceases in cattle susceptible to heat stress. With increased ambient temperate there is a corresponding decrease in volatile fatty acids (VFAs) in the rumen, mostly due to decreased production of both acetic and propionic acids, with propionate production more impacted (Kelley et al., 1967). The consequentially increased acetate: propionate ratio reduces the heat increment of VFAs, and therefore heat load (Bianca, 1965). Findings are not always consistent: contrary to other studies, there was no change in propionic acid, an increase in butyric and valeric acids, and a significant decrease in acetic acid (Nonaka et al., 2008). Rumen VFA levels were not affected.

The heat stress-induced reduction in feed intake slows the passage of digesta through the gut allowing improved digestion rates i.e. increased breakdown and uptake of the nutrients within the digesta especially of slow fermenting starches (Kennedy and Cronjé, 2005). For example, Young Holstein heifers subject to 14 days at 33°C experienced increased digestibility of gut contents due to decreased rate of passage of digest through the gut: gut retention time increased from 56 hours at 20°C, to 92 hours at 33°C (Nonaka et al., 2008). Heat stress also directly affects rumen motility independent of the reduced feed intake (Attebery and Johnson, 1969). In a controlled feeding experiment, amplitude, frequency and regularity of rumen contractions were decreased with exposure to 5 days at 38°C. Unfortunately, the extra energy obtained from increased digestibility does not adequately compensate for the 7 – 25% increase (depending on animals and conditions) in maintenance energy requirements during heat stress (Beede and Collier, 1986).

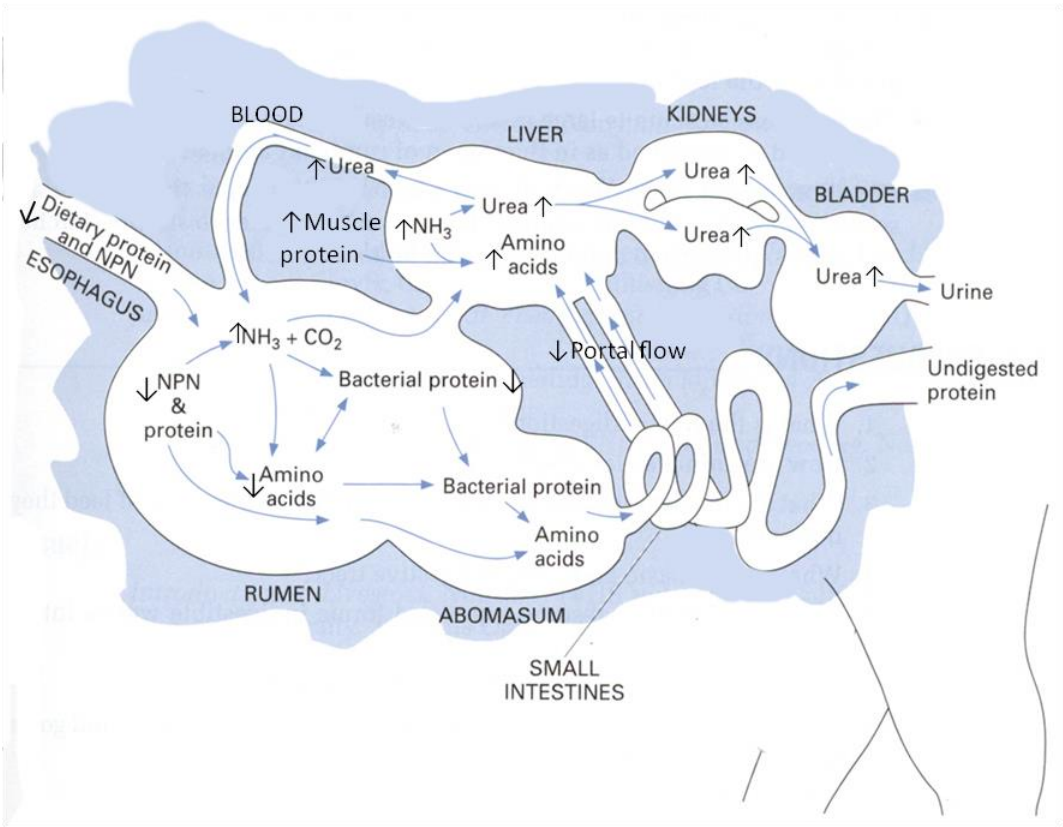

Figure 4.13 Schematic summarizing altered N metabolism in heat-stressed ruminants. (Adapted from Figure 16.9 Protein pathways in the ruminant. J. Bryant and B.R. Moss, Montana State University.)

However, various studies support or challenge the view that decreased rate of passage in heat stress improves digestibility. Bernabucci et al. (1999) concluded that digestibility under heat stress was not always dependent on DMI or altered rumen passage rate. In assessing the passage rates of digesta and digestibility at two different time points in heifers subjected to 40 days of heat stress, they found increased digestibility coefficients soon after the onset of heat stress, but no difference after 40 days of heat compared to the thermoneutral conditions. In contrast, the rumen passage rate was reduced at both heat stress timepoints. A follow-up experiment to assess the effect of different periods of heat on rumen function and digestibility was conducted in sheep (Bernabucci et al., 2009a). Rumen passage rates and digestibility coefficients were determined initially in thermoneutral conditions, then at three timepoints during 49 days of heat stress, and finally within 15 days of returning to thermoneutral conditions. In this experiment, there was no difference in digestibility between the initial thermoneutral state and shortly after the onset of heat stress. Digestibility decreased with ongoing heat stress and even after 17 days of thermoneutral conditions after the heat stress, digestibility had not returned to the pre-heat-stress levels. Rate of passage in the rumen did consistently decrease with time in heat stress, and mean retention time increased accordingly. The rate of passage in the caecal colon also dramatically slowed after 40-49 days of heat stress exposure. All the digesta movement parameters did not recover after 10-15 days return to thermoneutral conditions (Bernabucci et al., 2009a).

Additional evidence for perturbation of rumen function were the strong effects of increased ambient temperature on rumen ammonia levels and lactic acid production, both being increased initially, with slow rates of decline post-feeding (Mishra et al., 1970). Rumen pH and oxidative-reductive potential, a measure of microbial activity, were depressed. When the warm conditions were combined with a high grain-diet, there were additive effects on rumen pH and oxidative-reductive potential. Feedlot concentrate based diets can lead to subclinical or overt rumenal acidosis and increased osmolality of the rumen digesta due to increased acetate (Kennedy and Cronjé, 2005). In hot conditions, this is exacerbated by the loss of buffering capacity in the rumen from decreased saliva flows to the rumen due to panting and drooling. The lowered rumen pH also reduces fibre digestion as the participating microflora is most affected by acidic pH (Shearer, 2005).

4.4.4 Blood buffering and electrolyte balance

With the slow loss of water from the body in warm conditions, there is an initial increase in the concentration of ions, especially Na+, in the extracellular fluid in tissues and a smaller volume of water is retained. These changes stimulate drinking and reduce urine flow. For example, lactating dairy cows at 30°C will increase water intake by 1.3-fold to counter increased losses of water to urine (15%), sweat (~60%) and the respiratory tract (50%, due to increased respiration rate). Faecal water loss is reduced by about a third (Beede and Collier, 1986). As dehydration proceeds, electrolytes do not continue to increase in concentration in body fluid, as they are excreted from the body in roughly proportion to water loss. So, after the initial increase in Na+ excreted in urine (sourced from extracellular fluid), water is drawn from cells, which leads to an increase in K+ excretion in urine. Animals can therefore become depleted in both water and primary electrolytes

Thus, the combination of increased water loss through urine and perspiration, and reduced feed intake during heat stress, has implication for blood buffering, electrolyte balance and micro-mineral replacement. Increased losses of Na+, Mg+, Ca+2, Cl- (but not P) are highly correlated with sweat rate (Jenkinson and Mabon, 1973). An additional heat stress induced complication is respiratory alkalosis arising from sustained increased respiration rate and panting. The increased expiration volume drives CO2(g) from the blood by dissociation of dissolved carbonic acid (H2CO3) to CO2(g) and water. The consumed carbonic acid is replaced by association of HCO3- and H+. The alkalosis is caused by depletion of blood H+, thus increasing blood pH. The kidneys become involved by eliminating HCO3- which is accompanied by a cation, Na+ or Ca++. As a consequence of the increased urine HCO3- , urine pH also increases. Na+ excretion is further encouraged by reduced blood aldosterone levels of heat-stressed cows, in an attempt to preserve blood K+ (lost to excretion by sweating). Na+ excretion maybe 80% higher in heat-stressed cows compared to their thermoneutral rate of loss (Sanchez et al., 1994).

To add to the complexity of blood buffering during high heat load, renal conservation of H+ can promote a compensatory metabolic acidosis (Schneider et al., 1988). As animals cool overnight, and respiration rate and rectal temperature fall, respiratory alkalosis is relieved, but renal proton retention coupled with continued excretion of HCO3- induces a systemic acidosis, which in turned is countered by increased excretion of H+ (as NH4+) and conservation of HCO3-. Urine pH falls well into the early morning.

A further consequence of heat stress on blood buffering and electrolyte balance in heat stress is the altered rumen activity. Reduced gut motility and feed intake leads to increased fermentation to VFAs per unit of feed. The tendency toward rumenal acidosis is exacerbated by reduced buffering capacity due to reduced salivary flows. Chronic and subclinical rumenal lactic acidosis may ensue. The reduced blood flow to the gut mucosa is thought to impair absorption of essential elements, however only dietary phosphorus was shown to have reduced portal blood concentrations (~50%) when compared to thermoneutral cows on reduced rations (Sanchez et al., 1994).

4.4.5 Oxidative metabolism

Heat stress is known to induce deleterious systemic and localised tissue changes to the oxidative status. The major group of oxidative molecules are based on oxygen and known as Reactive Oxygen Species (ROS). They can be endogenous and/or exogenous reactive molecules or oxidising agents derived from normal metabolism, diet and the environment. ROS can be both harmful and beneficial. The major ROS are the superoxide ion, O2-, H2O2, and the highly reactive (extremely transient) hydroxyl ion, ·OH. An excess of ROS in cells can lead to damaged DNA, protein and lipids (reviewed in Valko et al., 2007; Buonocore et al., 2010). The consequences of increased oxidative activity for mammals are defects in reproduction including sperm function, embryo and fetal growth and placentation (Miller et al., 1993; Buonocore et al., 2010; Pourova et al., 2010) and contribute toward the development and progress of chronic diseases and cancer (reviewed in Valko et al., 2007; Pourova et al., 2010).

Cellular biochemical mechanisms maintain a balanced redox status between intracellular antioxidants and ROS. The antioxidants include enzymes such as superoxide dismutase, glutathione peroxidase, catalase and the glutathione transferases, and small molecules such as glutathione

(GSH), thioreoxidin, N-acetyl cysteine, pyruvate, NADPH, vitamin C (ascorbic acid), vitamin E (α-tocopherol), carotenoids and flavenoids (Valko et al., 2007). Glucose is also integral to maintaining redox status by ensuring that enough NADPH is supplied (through the pentose phosphate cycle) to allow the antioxidant enzymes to function (Lord-Fontaine and Averill-Bates, 2002).

Redox status is determined by measurement of plasma, tissue or cellular oxidant and/or antioxidant agents. Levels of the various antioxidants and the activities of antioxidant enzymes are commonly assayed, along with GSH and the GSH:GSSG ratio. GSSG is the oxidized form of GSSG, thus a low ratio is indicative of oxidative stress. Indications of lipid peroxidation (specifically PUFAs) due to ROS activity can be indirectly measured through the products of hydrolysis of lipid hydroperoxides. This reaction yields small acyl compounds, such as aldehydes (e.g. malondialdehyde), alcohols and hydrocarbons. Thiobarbituric acid reacts with malondialdehyde and similar compounds, thus the presence of thiobarbituric acid reactive substances (TBARS) is used as a general indictor of oxidative status with the caveat that this simple chemical assay has limited specificity and sensitivity (Buonocore et al., 2010).

Studies of the oxidative status of cattle during heat stress have returned a diversity of results. Dairy cows at various phases of lactation have received most attention, mostly using comparison between summer and winter oxidative status as measured by plasma, leucocyte and/or red blood cell (rbc) indicators. In two independent studies of lactating Holstein cows over summer and winter, Tanaka et al. (2007, 2008) found that summer conditions, relative to winter, were associated with decreased plasma ascorbic acid and sulphydryls (thiols) and increased TBARS. Rectal temperature showed a moderate negative correlation with plasma ascorbic acid levels ($r = -0.34$ and -0.445). Bernabucci et al. (2002) compared the oxidative status of Holstein cows prior to and after calving in spring or summer. The seasonal change was distinct enough to induce increased respiration rate and rectal temperature in the summer transition cows. There was no difference between the spring and summer cows in oxidative status as assessed by plasma markers, however the same markers in rbc, TBARS, thiols, glutathione peroxidase as well as superoxide dismutase activities were increased. The authors suggested that rbc are a more sensitive site for indications of oxidation and that the increased rbc enzymes and metabolites are compensating for the increased oxidation induced by heat stress. Trout et al. (1998) compared the oxidative status of thermoneutral cows relative to their heat-stressed counterparts which experienced a mean ambient temperature of 38.3°C for 10 hours daily. There was no discernible effect of heat stress on plasma antioxidants such as α-tocopherol, β-carotene, retinol and retinyl palmitate, nor muscle malondialdehyde and milk yield.

Lakritz et al. (2002) monitored the oxidative status of non-pregnant non-milking Simmentals cows in climate chambers over a period 14 day cycling 4 hourly between 26 and 33°C. Relative to thermoneutral controls, only rbc GSSG was increased, with no difference in total blood GSH or rbc GSH. Burke et al. (2007) followed the redox status of mononuclear blood cells (PBMC) sampled from pastured Angus cross heifers over the summer months. The time course revealed that as ambient temperature increased, the cellular GSH:GSSH ratio was lower, as was Se content and glutathione reductase and glutathione peroxidase activity. Different results undoubtedly reflect variation in experimental protocols, stage of lactation and breed. No studies have been performed in beef cattle thus far. It is most probable that redox balance is challenged at the cellular, organ and systemic levels in heat stress and possibly during recovery. The extent of the imbalance will depend on severity and period of hyperthermia which impacts the level of damage to the tissues and organs.

5 Reponses to hyperthermia by organs and cells

5.1 Gut and liver integrity, and muscle function

A major adaptation to heat stress is altered blood flow directing blood from the core to the skin. Along with increased peripheral vasodilation, the larger volume of cutaneous blood enables escalation of evaporative heat loss and decreased blow flood to internal organs (Beede and Collier, 1986). This adaptive blood shunting has major consequences for the viscera if prolonged. The metabolic stress due the affected organs and consequent tissue damage can trigger difficult-to-control and spiralling pathology in the hyperthermic animal. The limited studies in ruminants on these outcomes of heat stress has been addressed by referring to studies in model species (mostly rodent models) to ensure that the current knowledge of the cost of heat stress on the gut, liver and muscle is captured in this review.

5.1.1 Gut barrier disruption and damage to the gastro-intestinal tract

von Engelhardt and Hales (1977) demonstrated a reduction of about 30% in the blood flow to the mucosa of the reticulum and dorsal rumen in heat-stressed sheep relative to thermoneutral controls. An acute heat stress model in rats revealed decreased O2 saturation and pH and increased pCO2 in portal venous blood; these indicators of reduced blood flow persisted for 1- 2 hours after heat stress and after internal body temperature had returned to a normal range (Hall et al., 1999). Under these conditions, the jejunal epithelium was shown to be highly vulnerable to hypoxia. There was little effect of heat stress on the blood flow to the intestinal musculature and other tissues underlying the mucosal epithelium (von Engelhardt and Hales, 1977; Hall et al., 1999). Although Attebery and Johnson (1969) had shown that chronic heat stress reduced the amplitude and regularity of rumen contractions, and suggested that neural inputs had been altered in nonlactating Holstein cows.

While rectal temperature is high and with much of the blood circulation diverted to skin and respiratory functions, the resulting hypoxia and oedema of the gut can have deleterious consequences for gut barrier integrity. The altered oxidative status of the gut epithelium as a consequence of hypoxia and/or acidosis has been nominated as one of the main culprits in damaging the cells and the tight junctions. In a careful study of mesenteric and portal flux in heat-stressed goats, Wang et al. (2011) demonstrated that heat stress increased plasma anti-oxidants (sodium oxidase dismutase, glutathione peroxidase and catalase) in portal and mesenteric blood but the actual net flux decreased across the portal drained viscera and mesenteric drained viscera. They concluded that given the reduced blood flow from the gut, there is no sign of actual increase in production of antioxidants despite increased concentrations of these anti-oxidant enzymes and increased GIT permeability to bacterial endotoxins.

Detailed evidence of intestinal cellular and tissue damage has mostly emerged from rodent models applying acute and sub-acute heat stress (Lambert et al., 2002; Oliver et al., 2012; Liu et al., 2012; Smith et al., 2012; Hagiwara et al., 2011; Yu et al., 2011). Models using anesthetized rodents have been developed since many of the acute heat stress regimes cause up to 50% mortality. Experiments with conscious animals apply less severe hyperthermic stress. As might be anticipated, the state of

consciousness affects physiological response to heat stress; for example, anesthesia impairs thermoregulation by suppressing the sweating response (in rats at least; Zhao et al., 2010).

The gut epithelial cells of anesthetized rats subjected to internal body temperature of 42.5°C for 90 minutes developed vacuoles, and detachment of the microvilli with some cells sloughed off from the underlying basement membrane (see Figure 5.1; Lambert et al., 2002). When the intestinal loops prepared from untreated rats were bathed in culture media at 41.5-42.5°C, similar and probably accelerated damage occurred, regardless of the region of the intestine from which the loops were prepared (Lambert et al., 2002). Oliver et al. (2012) confirmed the intestinal cellular and tissue damage in a mouse model where the heat-stressed mice were allowed 30 minutes recovery after achieving internal body temperature of 42.5°C; that is, significant increase of intestinal permeability and damage to the duodenal epithelium. Hall et al. (1999) assessed the extent of hypoxia in the rat intestine one hour after heat stress (internal body temperature greater than 40°C for 50-60 minutes). Alongside reporting epithelial damage as described above, there was a 30% increase in hypoxia in the jejunum with lesser impact in the duodenum whereas the colon and all submucosal cells remained unaffected by hypoxia in this model.

The effect of heat stress on protein expression in the gut in the acute mouse heat stress model has also been attempted (Liu et al., 2012). In heat-damaged small intestine, there was increased expression of defence and some cellular and tissue structural proteins (dynactin, keratin and cytokeratin), and reduced expression of sugar-processing enzymes, (different) cell structural proteins and DNA repair enzymes. The pattern of expression is probably indicative of initiation of repair, although repair from heat stress in the small intestine of mice surviving internal body temperature of 42°C is highly compromised, when compared with the rapid repair underway in those only exposed to internal body temperature of 41°C (Liu et al., 2012).

In an experiment that might model chronic heat stress, conscious rats experienced two hours hyperthermia daily for 10 consecutive days (Yu et al., 2011). In this case, the jejunal

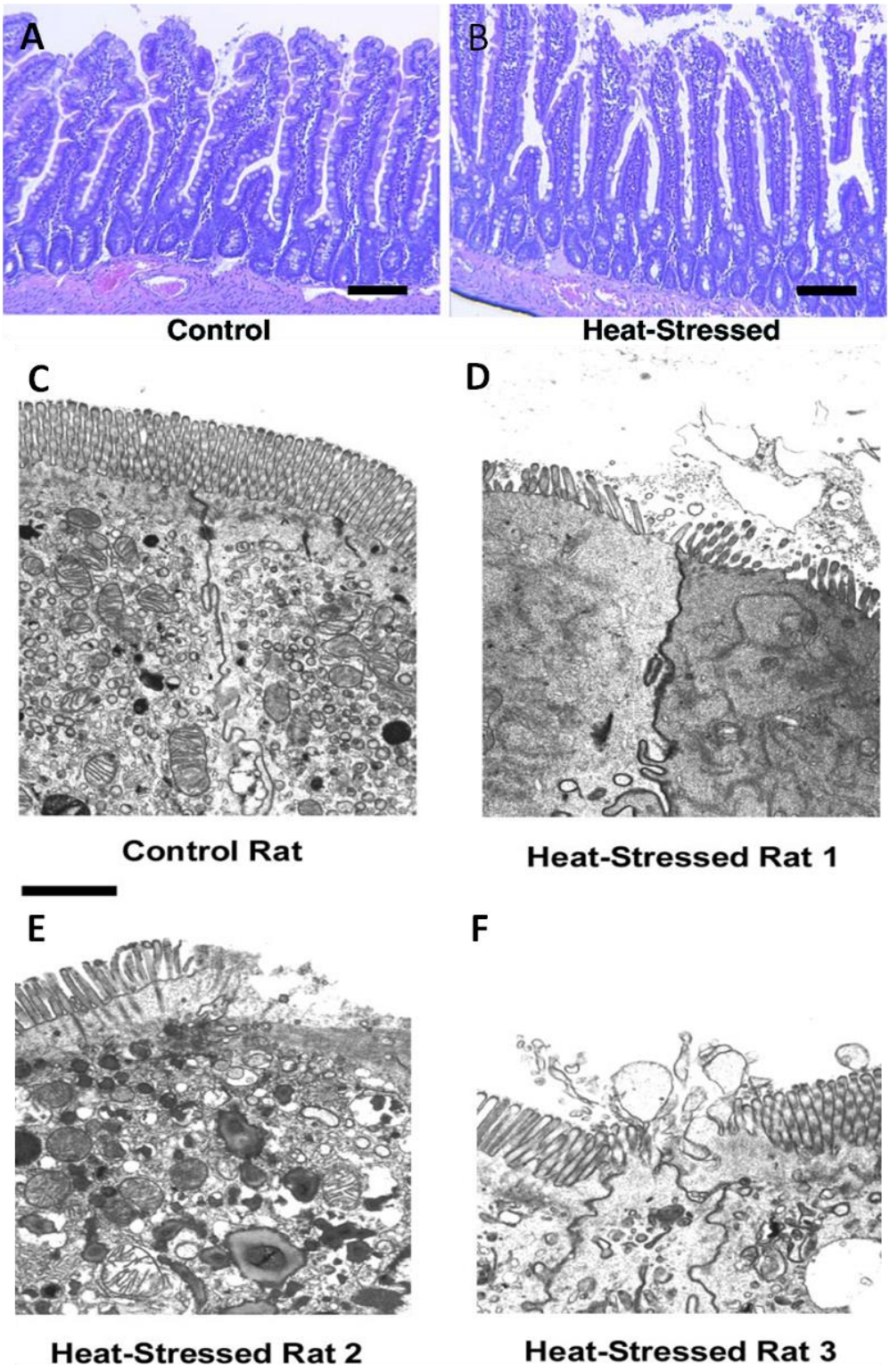

Figure 5.1. Micrographs of damaged intestinal epithelia from heat-stressed rats (adapted from Lambert et al., 2002). A and B: Light micrographs of stained jejunal intestine excised from a thermoneutral control rat and from a heat-stressed rat respectively. Note the loss of cells from the villi tips. C-F: transmission electron micrographs of the same tissue from a control rat (C) and heat-stressed rats (D-F). Note the disruption to the microvilli structure (D), loss of apical cell surface (E) and blebbing of the apical cell membrane (F).

epithelium suffered the most significant damage, with loss of epithelial cells and microvilli, and exposure of the lamina propria. Micro-array analyses of the mRNA transcripts of this tissue at day 3 indicated involvement of a number of pathways including increased HSP transcription, oxidoreductase activity, altered fatty acid and tryptophan metabolism, innate immune response and cytokine/adipokine signalling. The occurrence, nature and extent of heat stress damage in larger mammals is yet to be investigated; however, the presence of bacterial endotoxin in the portal and arterial blood of heat-stressed goats (Wang et al., 2011) suggest gut barrier dysfunction and thus gut epithelial damage.

5.1.2 Endotoxemia as a consequence of gut barrier disruption

As early as the 1970's, researchers had observed elevated circulating levels of bacterial endotoxins with heat stress, which implied breach of intestinal barrier (Oliver et al., 2012). It is now apparent that numerous psychological and physiological stressors and systemic inflammatory responses can induce changes to intestinal permeability in most species (Lambert, 2009; Mani et al., 2012; Oliver et al., 2012). Under normal conditions, the epithelial cells lining the gut and the various types of junctions between them effectively exclude passive uptake of molecules larger than 150 Da (Lambert, 2009). Non-specific transport of any class of molecule from the gut lumen to the circulatory blood system will occur if this barrier is degraded by opening of the intercellular junctions creating channels between the cells (paracellular spaces) or loss and damage of the epithelial cells (reviewed Lambert, 2009; Mani et al., 2012). Figure 5.2 summarises the consequences of heat stress on epithelial cells lining the gut.

Many studies have shown increased intestinal permeability and systemic endotoxin releases are coincident with heat stress and these two factors are seen as important contributors to morbidity and mortality of heat stress (Oliver et al., 2012). Endotoxin release provokes a systemic innate inflammatory response that may ultimately lead to multi-organ failure via pulmonary and cardiovascular failure (Lambert, 2009; Martich et al., 1993). The sequence of events is outlined in Figure 5.3. Release of digestive enzymes via damaged gut epithelium can also induce acute inflammatory responses in the gut. Activation of the inflammatory and immune responses will direct energy toward these systems and away from growth (Mani et al., 2012). Additionally, the febrile response associated with systemic inflammation requires a 13% increase in basal metabolism with a 1°C rise in internal body temperature (Kluger, 1978).

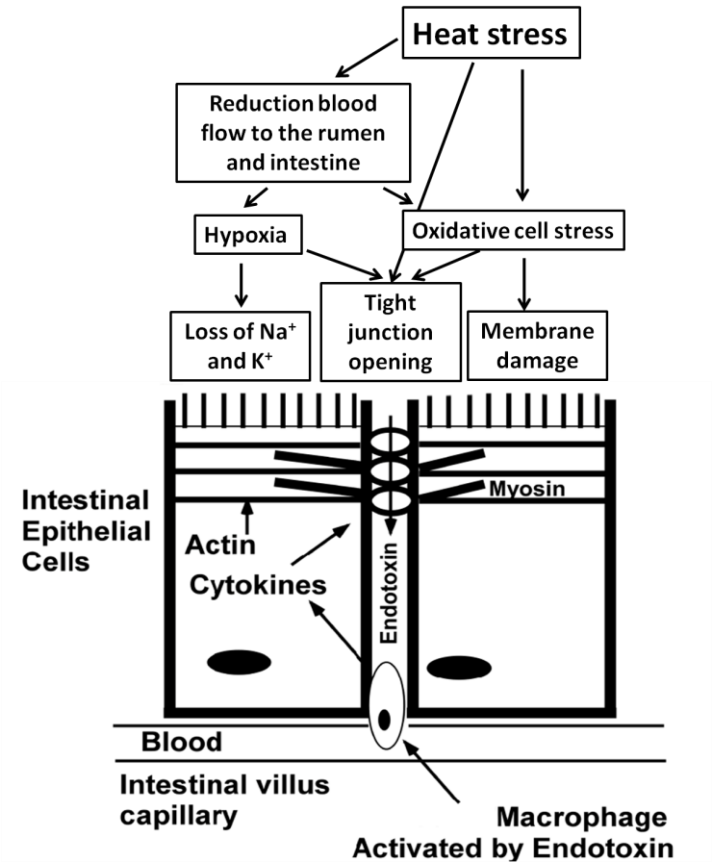

Figure 5.2. The effects of heat stress and reduced intestinal blood flow and on the intestinal epithelia. (Adapted from Lambert, 2004).

Elevated levels of circulating bacterial endotoxins are associated with reduced appetite, and altered absorption of amino acids and some sugars. As a consequence, skeletal muscle is catabolised to provide energy (Mani et al., 2012). Dietary factors are also implicated. Of relevance to cattle production: increasing the amount of dietary fat (especially emulsified fats) and highly digestible carbohydrate in ruminant diets are associated with elevation of circulating bacterial endotoxin, that is, rumen acidosis compromises the gut integrity, which allows microbial endotoxins into the local capillary beds (Kennedy and Cronjé, 2005).

In ruminants, the rumen is probably the most likely source of gut-derived bacterial endotoxin (Cronjé, 2005). A recent study of portal and mesenteric flows in dairy goats under heat stress has confirmed the portal drained viscera as the dominant contributor (80%) of gut lipopolysaccharide (LPS) entering the circulatory system during heat stress (Wang et al., 2011). LPS is the common name for the dominant class of endotoxin produced through degradation of the cell walls of Gram negative bacteria. By measuring blood flows and the plasma LPS in mesenteric and portal drained blood, LPS was evident in the mesenteric and portal bloods after just 4 hours of heat stress. It was detected in arterial blood after 10 hours of heat stress possibly indicating that the removal and detoxification processes of LPS by the liver was overwhelmed at this point. Over the 24 hours of heat stress, the mesenteric and portal bloods experienced approximately 200 and 300% increase in plasma LPS (Wang et al., 2011). The relationship between endotoxin release and heat stress in dairy and beef cattle remains unexplored but surely merits attention.

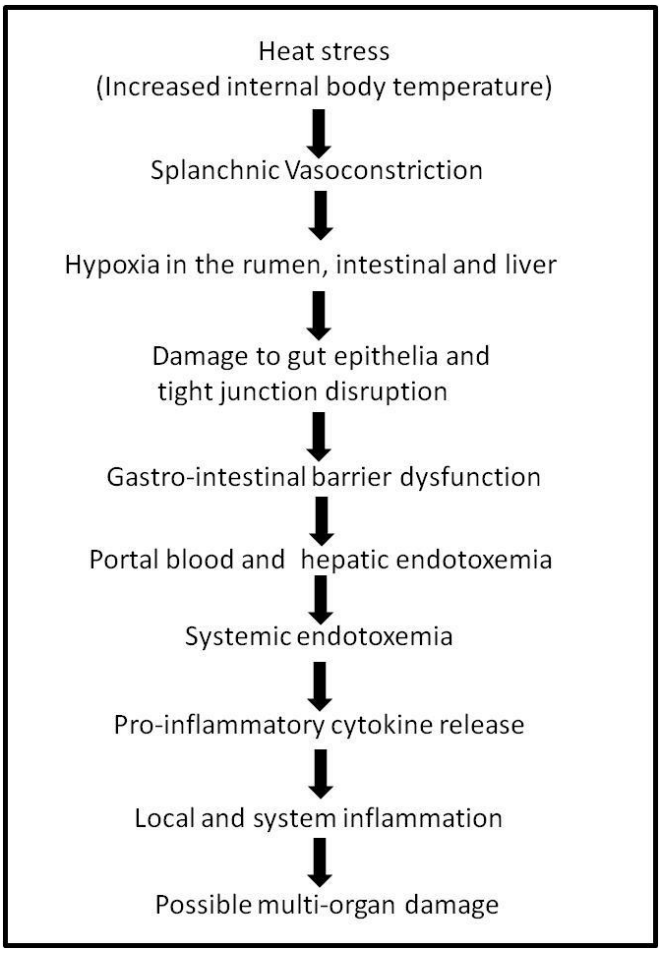

Figure 5.3. Consequences of heat stress on gut barrier integrity and barrier function. (Adapted from Lambert, 2004).

In ruminants, the rumen is probably the most likely source of gut-derived bacterial endotoxin (Cronjé, 2005). A recent study of portal and mesenteric flows in dairy goats under heat stress has confirmed the portal drained viscera as the dominant contributor (80%) of gut lipopolysaccharide (LPS) entering the circulatory system during heat stress (Wang et al., 2011). LPS is the common name for the dominant class of endotoxin produced through degradation of the cell walls of Gram negative bacteria. By measuring blood flows and the plasma LPS in mesenteric and portal drained blood, LPS was evident in the mesenteric and portal bloods after just 4 hours of heat stress. It was detected in arterial blood after 10 hours of heat stress possibly indicating that the removal and detoxification processes of LPS by the liver was overwhelmed at this point. Over the 24 hours of heat stress, the mesenteric and portal bloods experienced approximately 200 and 300% increase in plasma LPS (Wang et al., 2011). The relationship between endotoxin release and heat stress in dairy and beef cattle remains unexplored but surely merits attention.

5.1.3 Hepatic function

The release of liver enzymes into circulation is commonly observed in heat stroke victims and is indicative of hepatic damage. Significantly elevated aspartate transaminase and lactate dehydrogenase are associated with heat stress severity (Hashim, 2010). Increased plasma levels of hepatic alanine transaminase, aspartate transaminase and alkaline phosphatase are also detected in

acute heat stress models in rodents, which have been used to investigate the nature and extent of tissue and cellular damage in the liver.

In the acute heat-stress model used by Hall et al. (1999), the livers of the heat-stressed mice showed an overall 80% increase in hypoxia and 20% reduction in stored glycogen 1 hour after heat stress. Zone 2 hepatocytes and zone 3 Kupffer cells (hepatic macrophages) had over 100% elevation in hypoxia. Zone 3 hepatocytes, which are metabolically specialised in glycolysis, lipogenesis, and xenobiotic detoxification (using cytochrome P-450), displayed a 50% increase in hypoxia due to heat stress. Similarly, rat models of acute heat stress have revealed extensive hepatic damage with vacuolation in hepatocytes, and disrupted architecture involving the breakdown of sinusoidal integrity loss, neutrophil infiltration, and petechia (Hagiwara et al., 2011, Smith et al., 2012).

Comparing the livers of mice maintained at 34°C for 2 weeks with those kept in thermoneutral conditions, Bhusari et al. (2008) demonstrated that a wide range of metabolic changes were in play in the liver in response to mild but persistent heat stress. Most obvious was the 10% reduction in liver mass (for body weight) in the heat exposed mice. Survey of transcription in the heat-stressed livers found increased expression of anti-oxidant genes (including metallothionein) and energy metabolism genes. Assay of liver enzyme activity confirmed the increased activity of SOD, catalase and GPx. Of the genes involved in energy metabolism, there was increased transcription of the ATP synthetase □ subunit, pyruvate kinase (pyruvate production), stearoyl CoA desaturase (production of medium chain fatty acids), hydroxyl oxidase (oxidation of fatty acids) and a mitochondrial aldehyde dehydrogenase-2, but decreased ApoB which is involved in triglyceride formation and cholesterol transport. Genes involved in apoptosis and cell proliferation pathways were also upregulated. Surprisingly, there was no change in Heat Shock Protein (HSP) expression; although the proteosome □ subunit, involved in protein degradation was upregulated. There was no evidence of mitochondrial damage (Bhusari et al., 2008).

As described earlier, glucose metabolism in the liver is altered in heat stress. PEPCK, central to gluconeogenesis, appears to be specifically suppressed during heat stress in lactating cows, at least at the level of transcript expression (Rhoads et al., 2011). The suppression of PEPCK is not likely to be due to lack of substrate (oxaloacetate) since pyruvate carboxylase, the supplying enzyme is upregulated in heat stress most likely in response to reduced feed intake (Rhoads et al., 2011). Endocrine control of glucose metabolism in the liver is also altered in heat stress. Hepatic expression of Irs-1 (insulin substrate receptor -1) and a glucose transporter, Glut4, are both lower during chronic heat stress in mice (Morera et al., 2012). The fate of the main liver glucose transporter, Glut2, is not yet known. Compounding the apparent suppression of hepatic gluconeogenesis is the reduced expression of the growth hormone receptor (GHRc) and IGF-1 (Rhoads et al., 2010).

5.1.4 Skeletal muscle

Metabolically skeletal muscle appears to respond to heat stress with increased expression of the muscle glucose transporter, Glut4 (Morera et al., 2012). Altered mitochondrial metabolism was also inferred from increased pyruvate dehydrogenase kinase-4 transcription in an oxidative muscle (the soleus) when rats were exposed to 6 hours at 39.4°C (Sanders et al., 2009). Baumgard and Rhoads (2012) refer to reports of increased expression of pyruvate dehydrogenase kinase-4 mRNA in skeletal muscle of heat-stressed cows. These responses would indicate increased uptake and utility

of glucose by muscle in heat stress although to counter this line of evidence, there was no change in Glut4 gene expression in chronically heat-stressed sheep (Dunshea et al., 2012; unpublished).

Leakage of intracellular and tissue enzymes such as creatine kinase, aspartate transaminase and lactate dehydrogenase from muscle into blood after heat stress indicates skeletal muscle and possibly heart injury (Hashim, 2010). Further support for muscle damage in heat-stressed cattle is provided by observation of elevated plasma 3-methyl histidine in mildly but chronically heat-stressed lactating Holsteins relative to limit-fed (70% of ration) thermoneutral counterparts (Kamiya et al., 2006). The presence of 3-methyl histidine in plasma and urine is used as a measure of muscle catabolism. Heat stress induced damage to muscle would appear to recruit its involvement in the inflammatory response (Welc et al., 2012) and these changes described below. Interestingly, the response of antioxidant enzymes genes to heat stress in rats is possibly dependent on muscle type. The oxidative soleus muscle increased expression of a suite of these genes whereas the glycolytic tibialis anterior did not, although after a second exposure to the same stress the response in the soleus muscle was muted (Sanders et al., 2010).

5.1.5 Involvement of other organs

There are consequences from heat stress in other organs in terms of both tissue damage and their response. The reporting of these impacts are variable due to use of different heat stress models. Zhao et al. (2010) using an extreme heat stress model found that surviving anesthetised rats all displayed renal damage and active inflammation in the brain. In addition, inflammatory cytokines, TNF□, IL-1□ and IL-6, were all increased in all areas of the brain. Hagiwara et al. (2011) described oedema and inflammatory cell migration of the lung. Leon et al. (2006) did not detect major damage to brain, liver, lung, heart, or large intestine, but found renal damage and lymphoid necrosis in the spleen during the post-heat stress hypothermic phase in their mouse model. The spleen not surprisingly responds also with altered cytokine expression as outlined below.

5.2 Inflammatory responses

Inflammation is a protective, usually localised response to any insult to tissue and cells. Resident macrophages or macrophage-like cells recognise the tissue damage, and as the first responders, initiate a cascade of chemical signals that involve recruitment of other cells types to clean up and repair the injured tissue, and open up the capillary beds to allow fresh blood and cells into the site. If the injurious agent is not removed or not perceived to be removed, a chronic ongoing and non-healing inflammation can be established. Systemic inflammation occurs when chronic inflammation moves beyond the local site of acute inflammation and into blood vessel endothelium and organs. Heat stress invokes broad systemic responses including a systemic inflammatory response. Mounting evidence suggests that the first site of injury that invokes the systemic inflammatory response is the gut.

5.2.1 Inflammatory cytokines

Inflammatory involvement in heat stress responses and recovery necessarily raises the spectre of cytokine activity. Cytokines are diverse but generally small proteins with potent activity that are secreted by many cell types but especially by immune cells and cells with barrier function such as endothelia and epithelia. The effects and functions of cytokines are extremely broad and can be

executed at the local or systemic plane depending on the location of the secreting cells. Heat stress being a systemic condition, directly or indirectly, induces responses from almost every organs and tissue in the body. Many of the most acute and indirect responses are due to cytokines. A number of pro-inflammatory cytokines, interleukin-1 (IL-1, IL-1, IL-1 receptor agonist, IL-6, IL-10, INF and TNF are implicated in heat stress response with reports of elevated circulating levels in human heat stroke victims (Leon et al., 2006; Leon, 2007; Hashim, 2010).

In humans, plasma IL-1 and –1 have been correlated with severity of heat stroke (Helwig and Leon, 2011). In conscious rats subjected to acute heat stress (30 minutes, 42°C), circulating levels of IL-1 increased 10-20-fold, and serum IL-6 rose 100-fold in the 3-6 hours after heat stress (Hagiwara et al., 2011), whereas in anaesthetised rats increases in circulating IL-1IL-6 and IL-10 were far more modest immediately after acute heat stress (70 minutes, 43°C; Zhao et al., 2010). The dynamics of gene expression of IL-1 and –1 and their receptors in spleen and liver has been described for the 48 hours following heat stress in conscious mice, including an IL-1 receptor gene knockout (KO) line. In this model, both the wild-type mice and KO mice experienced a hypothermic 'overshoot' following the acute heat stress treatment. IL-1 and –1 and IL-18 gene expression in the spleen immediately responded to heat stress and remained high during hypothermia, returning to near normal at 48 hours recovery (Helwig and Leon, 2011). Hepatic gene expression was more subdued and appeared to lag behind the splenic response for these cytokine genes. The IL-1 receptor gene expression behaved similarly in both tissues, high at maximum internal body temperature and remaining high during the hypothermic phase. In the plasma, the two soluble forms of the IL-1 receptor exhibited different dynamics but were both still at relatively high levels at 24 hours post-recovery. The absence of the IL-1 receptor in the KO line appeared to improve thermotolerance, halving the duration of the hypothermic phase, time-to-recovery, and mortality (Helwig and Leon, 2011).

Elevated plasma IL-6 is the most consistently reported cytokine in human heat stroke and in an acute mouse heat stress model, raised levels of circulating IL-6 persisted during the 24 hour recovery period (Leon et al., 2006; Hashim, 2010). Persistent and high levels of plasma IL-6 in human victims and rodent models are correlated with severity and mortality; however, survival of IL-6 gene KO mice after passive acute heat stress was lower, implying a protective role (Leon, 2007). Exercised skeletal muscle is a known contributor to plasma IL-6 (reviewed Pederson and Febbraio, 2008). In making a connection to heat stress, Welc et al. (2012) found that the IL-6 gene expression in the skeletal muscle of heat-stressed anesthetized rats (that had achieved maximum internal body temperature of 42.4°C over approximately 3.5 hours) increased five-fold at 3 hours recovery. The circulating levels of IL-6 at 30 minutes and 2 hours recovery were 5-6-fold and 15-16-fold higher, respectively, than their thermoneutral counterparts. Cultured myotubes and myoblasts, generated from the mouse muscle C2C12 cell line, both responded to heat stress with 14- and 4-fold inductions of IL-6 gene expression, which continue to increase in the myotubes well into recovery (Welc et al., 2012). Reflecting the strong gene induction, myotube IL-6 protein secretion into the culture medium was increased two-fold. However, the strength of the induction was highly dependent on temperature, and in culture at 40.5°C, IL-6 gene expression in myotubes was not increased and was even suppressed during recovery.

There are many reports of increased circulating TNF as a consequence of heat stress. For example, both conscious and anesthetized rats subjected to acute heat stress experienced increases in serum

TNF (Hagiwara et al., 2011; Zhao et al., 2010). Welc et al. (2012) reported a four-fold increase in TNF gene expression in rat muscle after heat stress although plasma TNF rose only two-fold shortly after heat stress and quickly returned to normal levels. Nevertheless, involvement of TNF in the heat stress response and/or recovery is not so clear (Leon, 2007). It is possible that the TNF response is dependent on other factors such as LPS load and tissue damage. Leon et al. (2006) did not report a TNF response in an acutely heat-stressed and conscious mouse model that did not initiate observable gut damage. However, despite overt liver and intestinal damage, circulating TNF levels were not altered in fasted and anaesthetised rats subjected to acute heat stress (Smith et al., 2012), and Sonna et al. (2002) listed the TNF genes as one that is down regulated in heat stress.

A decade of studies using rodent models has started to unravel the role of cytokines in heat stress and stress in general. The contribution of cytokines to the heat stress response and recovery from heat stress is underexplored in many species. The release of inflammatory cytokines in ruminants will divert energy from growth, divert the immune system from its normal surveillance of environmental pathogens, and set up a febrile reaction to add to the heat load already accumulated by the animal.

5.2.2 Adipokines

Simplistically, adipokines are fat cell (adipocyte) derived cytokines with endocrine as well as inflammatory activities. The two best studied adipokines are leptin and adiponectin, which are pro-inflammatory and anti-inflammatory respectively (reviewed Wozniak et al., 2009; Conde et al., 2011). Leptin influences energy homeostasis via decreasing food intake, increasing fatty acid oxidation, decreasing lipogenesis in peripheral tissues and increasing insulin sensitivity. Adiponectin regulates feeding behaviour, decreases hepatic gluconeogenesis and glucogenesis, influences fatty acid oxidation in liver and muscle (leading to reduced fat stores), increases whole body insulin and modifies glucose metabolism by stimulation of pancreatic insulin secretion.

In a study using a mouse model, Morera et al. (2012) compared the gene and protein expression of leptin and adiponectin and their receptors in liver, skeletal muscle and adipose in chronically heat-stressed mice and mice maintained in thermoneutral conditions on 80% rations. Along the leptin axis and relative to feed restricted controls, both plasma leptin and expression of leptin in adipose increased in parallel with a marked increase in hepatic leptin receptor expression in the liver. Along the adiponectin axis, gene expression in adipose of adiponectin and its receptor in liver (Adiponectin receptor-2) were higher relative to feed restricted controls. In conjunction with many other heat stress results, heat-stressed mice had increased plasma insulin, with decreased plasma glucose and NEFA, and increased glucose clearance.

In the first report of adipokines in a heat-stressed livestock animals, sheep at the end of 21 days of daytime heat stress experienced increased gene expression of leptin and reduced expression of adiponectin in adipose (Dunshea et al., 2012; unpublished). Interestingly, a second experiment in sheep that extended to 4 weeks saw no heat effect on adipose leptin and adiponectin expression, possibly indicating acclimation.

5.3 Cellular and tissue responses

Attempts to dissect the systemic response to heat stress have inspired numerous studies using in vitro cell models such as culture of primary cells collected from animals or established cells from a variety of lineages and species. The most consistently observed components of the cellular heat stress response were summarised as compromising of altered nuclear functions with inhibition of DNA synthesis, transcription, and RNA processing and translation; arrest of the cell cycle; increased proteosomal and lyzosomal activities in response to increased accumulation of denatured, aggregated and degraded protein; cytoskeletal disruption; metabolic changes resulting in depletion of cellular ATP; and altered membrane permeability causing intracellular accumulation of cations (Na+, H+ and Ca+2) (Sonna et al., 2002). Most recently, induction and sensitivity of the endoplasmic reticulum stress pathway in response to heat stress has been demonstrated (Xu et al., 2011). Many of these responses are common to responses to other types of cellular insult. As might be anticipated with such an array of responses, numerous other proteins beyond the heat shock proteins and heat shock factor-1 participate in countering heat stress and in the recovery process. Amongst these are other transcription factors, antioxidant genes, adhesion proteins, growth factors, and interleukins. Many alter expression in a very tissue specific way and according to the severity of the insult (Sonna et al., 2002). Accordingly, the literature on cellular responses to heat stress is replete and diverse. A summary of a selection of these studies are presented based on two criteria: studies using cells derived from ruminants, or studies with established (mostly rodent) cell lines that contribute new and recent insights into cellular responses to heat stress.

5.3.1 Adipokine secretion

Bernabucci and colleagues interrogated the dynamics of leptin and adiponectin secretion by adipose cells and lymphocytes in response to heat stress. 3T3-L1 cells from the pre-adipocyte mouse cell line were matured into fat droplets containing cells and exposed to 24 hours at 37°C (normothermic), 39 or 41°C. The contrast in responses between the two hyperthermic temperatures and the two adipokines is remarkable (Bernabucci et al., 2009b). At 39°C, the cells increased adiponectin transcription by approximately 50% by 4 hours and stabilised at that level. Secretion of adiponectin lagged behind its transcription. Leptin transcription and secretion were unaffected. Culturing at 41°C reversed the response with adiponectin transcription and secretion virtually ablated while leptin expression was immediately elevated by 16-17-fold mean change over the 24 hour culture, although leptin secretion was increased by only 30%. With exposure of only 2 hours at 41°C, the resilience of these cells was revealed. Adiponectin and leptin expression and secretion were suppressed and stimulated, respectively, but recovered to near normal levels within 8 hours recovery in normothermic conditions (Bernabucci et al., 2009b). The implications for whole animal hyperthermia are the criticality of internal body temperature and amount of time at maximum internal body temperature. In this case, the 2°C difference in culture temperature induced diametrically opposed responses in the same cell line. These observations suggest that under different body temperatures, cells, tissues and whole organs can respond in very different ways.

The question of immune cell involvement in adipokine responses to heat stress was examined by Lacetera et al. (2009). Peripheral blood lymphocytes were collected from healthy Holstein cows and cultured for 65 hours under conditions mimicking circadian rhythm: a cycle consisting of 13 hours at 39°C (normothermic) followed by culture for 13 hours at a 39, 40, 41 or 42°C. The 42°C culture saw a

marked reduction in cell proliferation in response to the lymphocyte activator, Concanavalin A (but not viability) and a small decrease in leptin transcription. All temperatures above normothermic also caused a 10-20% reduction in expression of the leptin receptor (long form Ob-Rb). Thus it would appear that circulating lymphocytes are not contributing to the increased circulating leptin or response to it in heat stress.

5.3.2 Altered muscle fibre and metabolism

Of interest to growth and meat quality is the potential for altered muscle structure and metabolism in response to heat stress. Three day cultures of human primary skeletal myoblasts at 39°C promoted differentiation into large highly nucleated myotubes that were predominantly expressing type I myosin heavy chain which is associated with slow twitch oxidative muscle fibre type (Yamaguchi et al., 2010). Expression of myogenin, a muscle regulatory factor implicated in development to slow twitch muscle, was also increased in the cell culture under these conditions. The same analyses was conducted on the mouse C2C12 myoblast cell line and produced very similar results, however, when either culture was subjected to 41°C, myotube formation was severely disrupted (Yamaguchi et al., 2010).

Straadt et al. (2010) used the C2C12 myoblast cell line to monitor the metabolic recovery of differentiated myotubes over the 24 hours following heat stress at 42°C for 1 hour. Metabolism was most altered at 4 hours recovery and after 10 hours had not completely returned to normal. Recovery was driven by glucose, acetate, creatinine and several amino acids (leucine, lysine, phenylalanine and glutamine). There was increased uptake and consumption of glucose, while lactate, acetate and formate were removed from the cells to maintain intracellular pH. Uptake of amino acids into cells also occurred. The utility of glucose and increased anaerobic metabolism is consistent with hypoglycaemia and lactic acidemia often reported during heat stress in cattle (Baumgard and Rhoads, 2012).

5.3.3 Bovine mammary epithelial cells

The dairy industry has been long concerned with poor milk yields over the summer season and during heat stress. Furthermore, the effects of heat stress on the future potential milk production of high value dairy heifers are unknown. Primary culture of bovine mammary epithelial cells (BMEC) in hyperthermic conditions indicates severe consequences for ductal development (Collier et al., 2006b). Maintaining a BMEC culture for 7 days at 42°C saw rapid stalling and regression of the development of ductal branches. A micro-array analysis determined decreased expression of transcripts involved with lactogenesis and morphogenesis. In hyperthermic (1-2 hours) cell cultures, the responses to heat stress are rapid, with upregulation of DNA and protein repair stress-related genes and down regulation of cell cycle, metabolism, and structural protein genes. By 8 hours, culture cells expressed genes that were associated with apoptosis. Not surprisingly, cell growth was arrested after 24 hours of culturing. These preliminary data would indicate that mammary gland development is affected by heat stress and that follow-up studies in dairy heifers would be of value.

5.3.4 Intestinal cells and tight junction permeability

The association of gut ‘leakiness’ and increased circulating LPS in heat stress has motivated examination of the effects of heat stress on the formation of tight junctions between intestinal

epithelial cells in vitro. When cell monolayers of the mouse intestinal epithelial cell line (CaCo2) were incubated at 39 and 41°C, transepithelial resistance gradually reduced over the 24 hour hyperthermic period, while permeability across the monolayer increased (Dokladny et al., 2006). The altered permeability of the cell layer was not due to apoptosis, cell death or observable disruption of the tight junctions, but associated with changes in the level of expression of two tight junction proteins: a 2-4-fold increase in occludin and a decrease in Zona Occludin-1 (ZO-1) (Dokladny et al., 2006; 2008). Intestinal inflammation and permeability induced by S. aureus enterotoxin similarly saw a decrease in expression of ZO-1 in rat intestinal epithelium in vivo (Pérez-Bosque et al., 2006). Extrapolating this finding to the in vivo situation suggests that apparently moderate and physiologically relevant hyperthermia may cause subtle but deleterious changes to gut permeability. Reassuringly, when the intestinal cell monolayer was returned to normothermic conditions, tight junction function recovered within 4 hours.

In this experiment, Heat Shock Protein 70 (HSP70) expression was rapidly induced but the expression of claudin-3, another tight junction protein, remained unchanged. The expression of occludin would appear to be under control of Heat Shock Factor-1, the transcription factor that controls the heat stress response of HSP70. This transwell culture of the CaCo2 cell line may provide a useful model for assessing mechanism of hyperthermic stress and repair of intestinal cells.

5.3.5 Heat shock proteins

Damage to protein complexes within the cell, especially the cytoskeleton and nuclear matrix proteins, is a major consequence of heat shock (Gabai and Sherman, 2002). The cell responds with the production of protein with the specific functions of refolding, disaggregating, and degrading the damaged protein complexes. The dominant family of participants are the Heat Shock Proteins (HSPs) and their transcription factors, the Heat Shock Factors (HSFs). Collier et al. (2008) described the HSFs as the ‘first responders’ to heat shock. HSF-1 in particular induces gene expression of the HSPs, as well as coordinated changes to carbohydrate metabolism and cytoskeletal repair within the cell. Of the HSPs, the HSP70 and 90 families are clearly induced by cell heat shock and have been most closely studied in this context. However, HSP induction and synthesis is not peculiar to heat stress; their expression is influenced by a number of endocrine and immune factors, and contrary to their name, they are utilised in almost all cells in all tissues all the time. The HSPs are mainly ‘housekeeping’ proteins which are assigned the important work of ensuring ‘tidy’ protein complexes and ‘hygienic’ turnover and removal of damaged proteins ‘beyond their use-by date’. The significance of these functions is underlined by the fact these families of proteins are evolutionarily ancient and are found in bacteria through to plants and all animals. Some HSPs are secreted from cells in some conditions and by unknown pathways. The function of the circulating forms of HSPs is not understood.

The HSFs and HSPs have been extensively investigated in all the model species and humans, and now to some extent in livestock species. In beef and dairy cattle, it was hoped that the HSPs would enable development of indicators for heat stress and/or recovery/resilience and that genetic polymorphisms would identify individuals with improved thermotolerance. A recent 110 day study explored the relationship between HSP70 expression and body temperature (Gaughan et al., 2013). They reported that there was a strong relationship between HSP70 concentration and ambient temperature ($r^2 = 0.86$; $P < 0.0001$) and HSP70 concentration and photoperiod ($r^2 = 0.94$; $P <$

0.0001), but no relationship with core body temperature (r2 = 0.06; P < 0.0001) (Figure 5.4). This is clearly an area for on-going research.

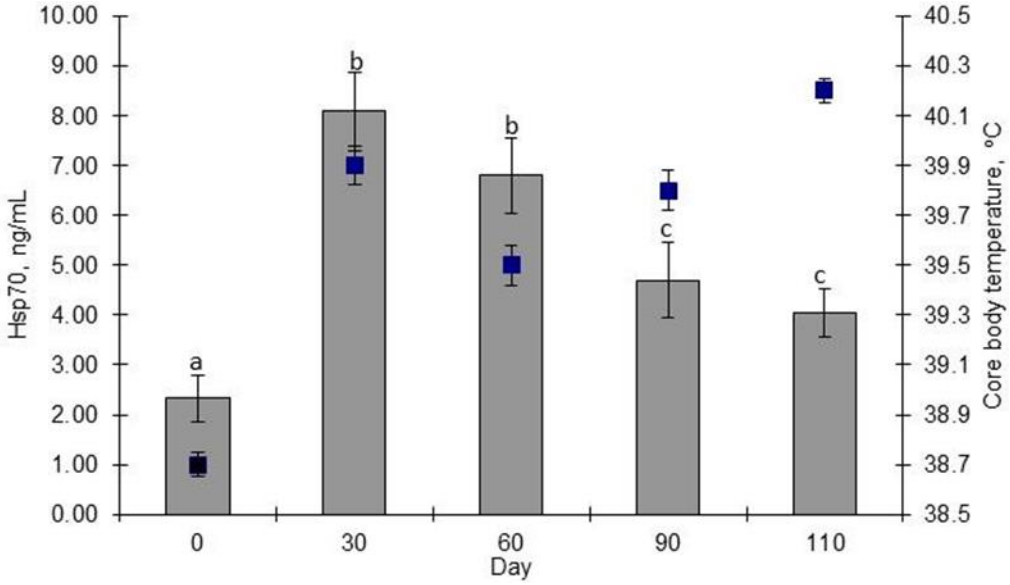

Figure 5.4. Mean (± SE) for HSP70 (ng/mL) concentration (bars) and core body temperature (oC) (■) on d 0, 30, 60, 90 and 110 of the study. Bars without a common letter differ (P < 0.05).

6 Nutritional interventions

Given the major response of a reduction in feed intake – either a reduction in voluntary feed intake or a reduction in the ration size offered to cattle – a common strategy is to increase the energy density of formulated rations to at least partially compensate for the reduced intake. This makes sense from an energetic and heat increment point of view, but is not without risks. The higher panting rate of cattle exposed to high temperatures sets up conditions for respiratory alkalosis and compensatory acidosis, subsequently reducing the capacity to buffer against the acidic products of rumen fermentation, which increases the risk of ruminal acidosis. In addition, another response to heat stress is a reduction in rumination due to fewer rumen contractions, a response exacerbated if ration formulation increases the content of energy-dense ingredients by replacing ingredients that provide 'physically effective fibre' (Mertens, 1997). Reduced chewing and rumination means less saliva reaches the rumen, which increases the risk of ruminal acidosis. A third risk factor to ruminal acidosis is the increase in drooling by heat-stressed cattle, which further reduces the amount of saliva buffer reaching the rumen.

Therefore, careful management is required to balance the desire to control heat load, maintain growth, and increase the energy content of the ration to compensate for a reduction in feed intake. The strategies in practice and still under development are outlined below. The first is to manipulate feeding quantity and timing, the second is to alter fibre: concentrate and fat ratios, the third is to increase the energy density of the ration by using ruminally-protected fats rather than increasing the grain content of the diet, and lastly to supply buffers (e.g. bicarbonate) in the ration.

6.1 Heat increment and altering the fibre, concentrate and fat ratios

Heat production increases with digestion and metabolism. This is known as heat increment. Heat increment is defined as "the increase in heat production following consumption of food by an animal in a thermoneutral environment" (Conrad, 1985). Heat increment includes the heat of fermentation, energy expended in digestion and the heat of nutrient metabolism (Conrad, 1985). Heat increment can be thought of as energy that must be dissipated from the animal. This is not usually a problem under thermoneutral or cold environmental conditions. However, under high heat-load, in which the animal's ability to dissipate body heat is impaired, additional body heat may be detrimental to the animal's wellbeing. Feed ingredients differ in heat increment, largely because of differences in the efficiency of utilization of the nutrient or the end-products of digestion (West, 1997).

Fibre has a higher heat increment than concentrates because of the lower efficiency of utilization of acetate relative to that of propionate and glucose (Baldwin et al., 1980) and the heat of microbial fermentation (West, 1997). There is considerable variation within these broad groupings (Table 6.1). For example, carbohydrates such as cellulose have higher heat increments than soluble carbohydrates such as sugar and starch (Conrad, 1985). The heat increment of a feed ingredient also depends on whether cattle are fed at, above or below maintenance. It is possible to formulate diets according to heat increment but evidence on whether this practice is effective in alleviating heat stress in feedlot cattle is inconclusive.

The concept of physically effective neutral detergent fibre (peNDF) has been proposed to explain the effectiveness of the NDF in promoting chewing (Mertens, 1997). The peNDF is related to the physical characteristics of fibre such as particle size. If the summer ration for cattle is re-formulated to increase the energy density, to partially compensate for the expected decline in feed intake associated with heat stress, and to reduce the fibre content to minimise heat increment, then it becomes important that the fibre that is used has a high content of peNDF. That is, whilst there is a case to reduce the overall fibre content of the diet in preparation for high ambient temperatures, it is critical that cattle consume a ration that triggers sufficient chewing activity to minimise the risks of ruminal acidosis. There is a large range in the amount of chewing activity for each unit of NDF across different hays (from 111 to 209 minutes chewing activity/kg of NDF; reported in Mertens, 1997). This is because the NDF fraction is a non-uniform fraction with various pools that differ in digestibility (Raffrenato and Van Amburgh, 2010), creating an opportunity to not only modify the concentration of NDF in the ration, but the digestibility of the NDF (because digestibility of NDF can vary independently from the NDF concentration of a feedstuff). For example, the 'indigestible NDF' pool can range from about 25% of the total NDF fraction for grasses and corn silage, to around 35% for straws and hays, and over 40% for lucerne (Raffrenato and Van Amburgh, 2010).

Further work is required to test the 'physical effectiveness' of different fibre sources used in Australian feedlot rations. Research is needed to identify the best fibre sources, the most appropriate processing (as chopping length can also have a considerable effect on chewing activity), and inclusion rates that achieve an appropriate balance between (i) reducing heat increment and increasing the energy density of the ration and (ii) maintaining chewing activity to promote saliva that helps buffer against ruminal acidosis. The best strategy may be to design a ration with a relatively low total NDF content, thereby reducing the heat increment, but with a high fraction of the NDF being indigestible (to promote chewing and buffering). This hypothesis needs to be tested,

especially considering the changes in the rate of passage and rumen fermentation associated with heat stress (see section 4.4.3).

Dietary fats have the lowest heat increment, followed by carbohydrates and then proteins. Dietary fat has a low heat increment relative to acetate because of greater efficiency of utilization (Baldwin et al., 1980). High efficiency of utilization results in lower heat production, and therefore reduces the impact of environmental heat load. Use of fat or low heat increment ingredients for ruminants is limited because diets containing more than 5% to 7% total fat suppress rumen function unless presented as in a rumen protected form. (See section 6.3 for an expanded discussion).

High-concentrate diets with little roughage result in a low rumen pH and a shift in rumen bacterial populations (Van Soest, 1994), which may result in changes in biohydrogenation pathways (Leat et al., 1977). West (1997) commented that although dairy cows may not show signs of reduced heat stress when fed high-fat diets, they will benefit from the greater energy density during periods of depressed DMI.

Table 6.1. Average heat increment (MJ ME/kg) of various feed components.

Feed component	**Heat increment**
Fishmeal	0.47
Vegetable oil	0.45
Animal fat (tallow)	0.25
Protein	0.52-0.55
Concentrate (100%)	0.52
Roughage (10%)	0.70
Grain, rapid fermentation (wheat, barley)	0.46
Grain, slow fermentation (sorghum, maize)	0.26-0.42

Source: Blaxter, 1989; NRC, 1996.

In taking a different approach, diet choice experiments can be an insightful way to gauge if a particular diet or feed ingredient provides a net benefit or cost to animals. There is a large literature base supporting the notion that animals modify diet selection in response to ‘metabolic discomfort’ (Forbes and Provenza, 2000). That is, the consumption of foods leads to negative or positive feedback signals from the gastrointestinal tract, liver and other cells of the body, which are transferred to the brain via nerves, neurotransmitters and hormones. In this way, diet selection is an integrated response to the consequences of consuming a food. Passini et al. (2009) offered cattle a choice of roughage (sugar cane and urea) or a concentrate feed under either thermoneutral conditions or thermal stress (38°C). The animals reduced voluntary intake but did not alter the proportion of roughage:concentrate in their self-selected diet. The lack of change in diet selection (22% roughage in the selected diet in both thermal comfort and stress) suggests that there was no advantage in increasing or decreasing the proportion of roughages. The prime response of animals to thermal stress is, therefore, a reduction in feed intake as this can have a marked and immediate reduction on the heat increment of eating, whereas a change in diet composition has more subtle effects.

In a study with Awassi lambs in Turkey, Kaya, (2001) used a full cafeteria feeding system that allowed for a greater degree of diet selection. It was found that the biggest change in diet selection in response to being reared outside under a higher THI (compared to cooler indoor housing) was a

significant increase in the proportion of full-fat soybean and wheat bran in the overall diet of the lambs, and non-significant reductions in the proportion of barley grain, cottonseed meal and lucerne hay (Figure 6.1). These data imply that the animals received positive feedback by increasing the selection of high-fat dietary components when exposed to higher outdoor temperatures. Results from Zulkifli et al. (2007) with chickens support the notion that animals will self-select a higher proportion of a high-fat feedstuff in response to thermal stress. Interestingly, the chickens preferentially selected a palm oil diet rather than soybean diet, consistent with the idea that a mono-saturated fat has a lower heat increment than polyunsaturated oils.

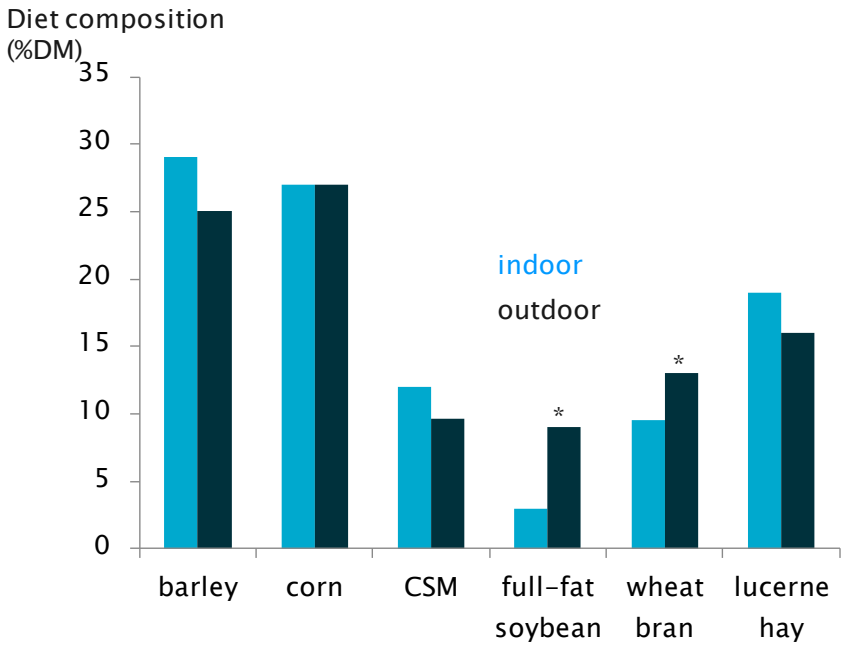

Figure 6.1. The dietary proportion of different ingredients offered to Awassi lambs in a cafeteria feeding system housed outside or indoors, with the outdoor reared animals exposed to a higher THI during the morning (from Kaya, 2001).

Providing animals with the opportunity to select particular ingredients is not practical in commercial feedlots, but there is the potential for ration formulation to be modified based on diet selection studies in an attempt to reduce the impact of high ambient temperatures on DMI. Collectively, the data from diet selection studies suggest that modification to the ingredient and macronutrient composition could be an effective strategy under mild heat stress, but less so as practical strategy under more severe conditions of high and continuous exposure to high temperatures when a reduction in daily feed intake becomes the necessary adaptive response. This point is particularly evident from the studies of Pacetti, (2006) who showed that the biggest dietary effect on body temperature was not by creating a low 'heat increment diet' by replacing silage and hay with cottoned meal and Megalac®, but by restricting feed intake below ad libitum quantities (Figure 6.2). Restricted feeding concepts are explored later in the discussion on "Feeding Strategies".

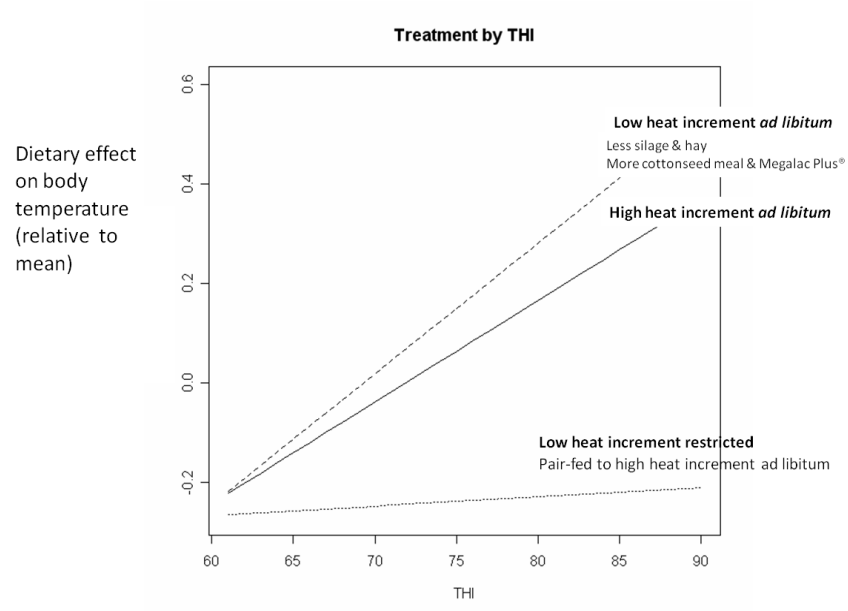

Figure 6.2. The effect of diet composition or feeding level on body temperature across a range of THI (temperature humidity index) values (Pacetti, 2006).

6.2 Supplemental dietary protein

Reduced DMI during hot weather reduces energy intake and furthermore, energy is diverted from growth to maintenance because of increased physiological demands. This may result in an excess of protein in the diet relative to energy intake. An excess of protein may contribute to reduced efficiency of energy utilization because energy is used to convert excess ammonia to urea (West, 1999). Therefore, it may be necessary to reduce the protein component of the diet during periods of high heat-load or to increase the energy density of the diet. However, data derived from dairy cows suggest that declining DMI intakes will result in a negative nitrogen balance and therefore more dietary protein will be required (West, 1997). Much of the dairy work has been carried out using diets or pasture with high crude protein contents (>20%). It is clear that a balance between rumen degradable protein and rumen escape protein is required. The dairy research suggests that rumen degradable protein should not exceed 61% of total crude protein (Huber et al., 1994). The key factors appear to be a balance between dietary protein and energy, and protein quality. This is clearly an area where research is needed.

Given indications that muscle catabolism was increased as a response to heat stress, the effects of manipulation of dietary crude protein in heat stress has been studied. Huber et al. (1994) in reviewing the literature, to that time, found that the percentage crude protein, protein digestibility and protein quality had no effect on respiration rate or rectal temperature in heat-stressed lactating dairy cows. It must be noted that dietary crude protein is high in US dairy feeds, often approaching 20% DMI. Hassan and Roussel (1975) showed DMI increased by 11% and milk yield by 6.5% as well as increased milk protein in heat-stressed cows feed 21% crude protein vs. 14% crude protein. Other benefits of the higher protein diet were a slight reduced RR, increased blood glucose and haemoglobin values. On the other hand, Huber et al. (1994) obtained decreased DMI and milk yield along with increased PUN in a 18% crude protein diet with high protein solubility.

Rather than suggesting an increase in dietary protein, Ames et al. (1980) argued for its reduction, hypothesising that since ADG is reduced in heat stress due in part to increased energy requirements for maintenance, that the efficiency of protein uptake would be higher and ADG not affected by reducing dietary protein. These researchers calculated the protein content of rations based on ADG expected at ambient temperature at the time of feeding (twice daily). Both the protein-adjusted group and control groups consumed diets of same metabolisable energy. In two large trials with heifers and steers, there was no difference in ADG in controls vs. protein-adjusted diets regardless of mean ambient temperature. Overall, the protein efficiency ratio significantly increased for those animals on the protein-adjusted diets (Ames et al., 1980).

6.3 Supplemental dietary fats

Adding high energy supplements to diets has been investigated and used as a means of addressing negative energy balance in dairy cows (van Knegsel et al., 2007; Chilliard, 1993, Jenkins, 1993). Generally, lipogenic diets which are delivered by addition of supplementary dietary fat are known to partition more energy into milk production (van Knegsel et al., 2007; Chilliard, 1993; Jenkins, 1993). Under normal production conditions, supplemental dietary fat increased milk fat yield; associated increases in long chain fatty acid levels and mobilization of fat from adipose into milk results in reduced loss in BW and body condition. However, there is much variability arising from the nature of the additional lipids, the nature of the basal diet and the physiological status of the animal (van Knegsel et al., 2007; Chilliard, 1993, Jenkins, 1993). The source of the fat supplement did alter the FA chain length distribution of the milk fatty acids and their saturation (Moody et al., 1971). A consistent adverse outcome of dietary fat supplementation is reduced DMI. Many reasons are proposed for this effect including the susceptibility of the rumen microflora to direct and indirect toxic actions of excess dietary lipids in the rumen and intestine (Jenkins, 1993), decreased ruminal fibre digestion leading to extended gut fill, and that unsaturated LCFAs reaching the rumen invoke appetite controlling neuropeptides CCK and glucagon-like peptide (Drackley et al., 2003).

Not surprisingly, increasing dietary energy density is proposed as one means of overcoming the decreased nutrient intake caused by heat stress (Beede and Collier, 1986). The added advantage of supplemental dietary fats is the small heat increment on digestion. Additionally, there should be increased efficiency of long chain fatty acid (LCFA) incorporation into milk and adipose since these do not need to be formed de novo from acetate (Drackley et al., 2003). Early studies on the use of supplemental dietary fat in the diets of heat-stressed dairy cows noted little effect on DMI, BW or milk yield (Moody et al., 1967).

While rumen microflora can tolerate the addition of 3-5% dietary fat, the development of ruminally inert or protected fats such as calcium salts of fats or fats aggregated with denatured protein (protected fats) has allowed increased proportions of dietary fat supplementation (Beede and Collier, 1986). Thus studies in the 1990's appraised the use of ruminally inert fats such as "protected" or prilled fat preparations or the calcium salts of fats as dietary supplements in diary production and in moderating the effects of heat stress. Nevertheless, the inclusion of dietary fats in dairy cow diets has been found to reduce heat load in some studies (Beede and Collier, 1986; Huber et al., 1994; West, 1999). However, others have shown little benefit from added dietary fat (Knapp and Grummer, 1991; White et al., 1992; West, 1997; Chan et al., 1997). The disparity between published studies may be a function of differences in the type of diets fed.

In a more recent example, Drackley et al. (2003) compared the effects of a high carbohydrate diet to a high (3%) animal fat diet on lactating primiparous and multiparous cows in warm conditions. The high fat diet moderated respiration rate and rectal temperature, and increased plasma NEFA, and overall, showed a small advantage in the energetics of milk production over control cows on high concentrate diets. On the other hand, Moallem et al. (2010), likewise comparing a high fat diet (1% supplemented calcium salt fats) and a high grain diet on lactating Holsteins observed that heat stress depressed DMI in all groups (including control diet) and that the high energy diets caused further depression of DMI. The cows on the high fat diet showed the highest rumination rate, milk fat and plasma NEFA, but also the highest rectal temperature and respiration rate. In contrast, the high grain group had the highest plasma glucose levels and gained body weight. In accordance with early studies, Moallem et al. (2010) concluded that increased dietary fat goes to milk production, whereas increased dietary grains goes toward body stores, much as seen under thermoneutral conditions.

Some studies have focused on dietary supplementation with specific lipids subsets. Conjugated linoleic acid (CLA) and saturated fatty acid supplements have been investigated as possible ameliorants to heat stress in dairy cattle (Moore et al., 2005; Liu et al., 2008; Wang, J.P. et al., 2010). The rationale for the use of CLA was based on several studies that had shown that CLA in diets cause milk fat depression. The ensuing hypothesis being that CLA assists in diverting the energy of milk fat synthesis toward increased milk yield (Moore et al., 2005). Supplementation with CLA was compared to supplementation with palm oil derived fatty acid on lactating dairy cows over summer. The three week dietary treatments did not result in different respiration rate, skin temperatures, DMI, milk yield or other milk parameters, except for milk fat yield and % which were both reduced by ~30%. As anticipated, the CLA supplement also induced a shift in milk fatty acids from shorter chain LCFAs to longer chain LCFAs and increased total CLA in milk. Thus despite the theoretical availability of an extra 14.6 MJ of energy through milk fat synthesis depression, there was no obvious improvement of physiological status under heat stress.

In contrast, Liu et al. (2008) reported that after two weeks on the CLA supplemented diets, lactating cows did experience an average 0.6°C reduction in rectal temperature. After 4 weeks on these diets, plasma T3 level increased, and there were indications of decreased muscle breakdown, improved blood buffering and conservation of electrolytes. Wang, J.P. et al. (2010) specifically investigated the use of saturated fatty acids as supplemental dietary fat because they have less negative influence on bacterial plasma membrane function (Jenkins, 1993). Working in lactating Holstein cows, supplemental saturated fatty acids as a partial replacement for corn did not affect dry matter intake, but improved milk yield and milk fat content and yield, and reduced peak rectal temperatures in mid-lactation heat-stressed dairy cows (Figure 6.3). Plasma NEFA was decreased by 8%, but plasma glucose and hydroxybutyric acid were not affected by diet.

The use of fat supplements has been extended to beef cattle. 0'Kelly (1987) compared growing steers on low (2.5%) and high (9.2%) fat supplement diets in thermoneutral and heat stress conditions. Relative to the steers on the low fat diets, the steers on the high fat diets in both conditions, experienced reduced rectal temperature l and urinary N excretion. DMI in all groups was not different. However, the reduced plasma level of most lipid classes regardless of diet indicated evidence of heat stress induced perturbation to either lipid absorption and/or metabolism. Since faecal fat excretion was increased in these heat-stressed steers, O'Kelly (1987) pointed to mal-absorption as the cause of the depressed lipid plasma levels. White et al. (1992) also assessed the

treatment of growing steers to diets with and without supplemental fat and different protein sources. ADG and DMI were not affected by supplemental fats in diets in thermoneutral or warm conditions. Decreased total rumenal VFAs indicated inhibition of fermentation. Plasma urea N increased linearly with %fat in warm ambient temperature. The authors concluded that supplemental dietary fat was of little benefit to finishing steers regardless of ambient temperature.

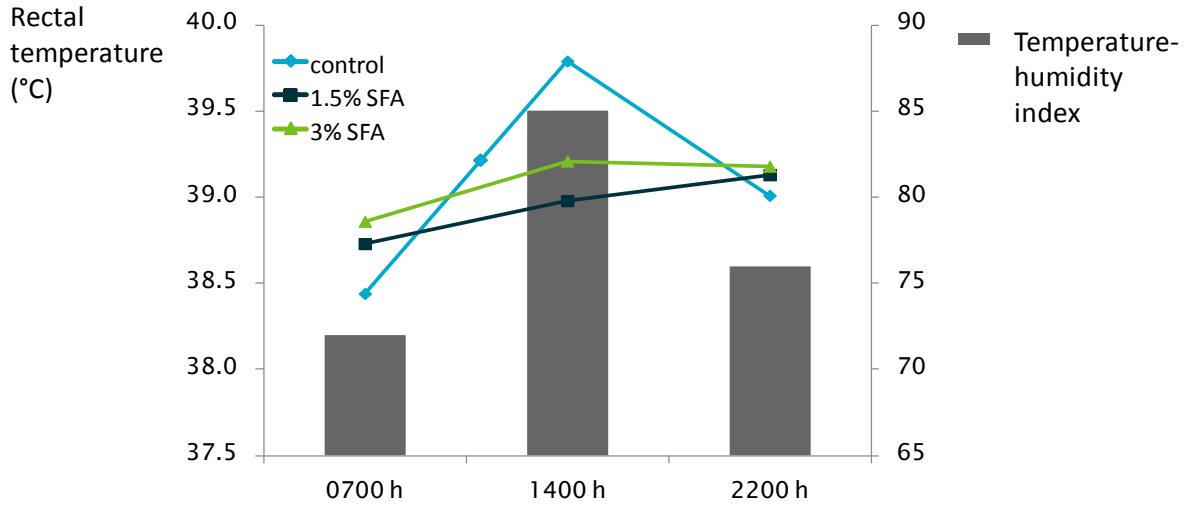

Figure 6.3. The effect of dietary supplementation with saturated fatty acids on rectal temperature of heat-stressed mid-lactation dairy cows (Wang, J.P. et al., 2010).

In more recent beef cattle studies, steers exposed to high heat-load and fed grain diets high in Na+ and fat (0.56% and 5.1%, respectively) had lower DMI than those feed a low Na+, low fat diet (0.19% and 2.9%, respectively; Gaughan and Mader, 2009). The inclusion of dietary fat coupled with cooling has shown a positive response: ME intake was 23% greater in Bos taurus steers exposed to high heat-load conditions and fed a diet containing 6.3% fat than steers fed a diet containing 2.1% fat when steers were cooled during the day (Gaughan et al., 2008a). Core body temperature was also significantly lower for steers fed the high fat diet compared with those fed the low fat diet, irrespective of cooling. In summary and as a general guideline, West (1997) suggested that no more than 30% to 40 % of total dietary fat should come from whole oil seed (saturated oils), 40% to 45% from other basal ingredients, and 15% to 30% ruminally inert fats. Gallardo et al. (2001) suggested that the characteristics of a “cold” diet are higher energy content per unit volume, highly fermentable fibre, low protein degradability and high bypass nutrient content. These recommendations are useful for feeding totally mixed rations.

6.4 Electrolytes and buffering mineral salts

As previously described, respiratory alkalosis induced by increased respiration rates and the compensatory metabolic acidosis challenges the regulation of blood acid-base balance. Supplementation of feed with buffering mineral salts containing the Na+ and/or K+ salts of HCO3- and/or CO3-2 have seen some reports of success in improving reduced feed intake and milk production in heat-stressed cows. However, despite widespread use in the USA, there is no conclusive and consistent evidence of the effectiveness of this supplementation in dairy cattle and

very little research as to its application in beef cattle. In meta-analysis of 15 dietary supplementation studies by a number of researchers, Sanchez et al. (1994) found increasing dietary availability of HCO3- and/or CO3-2 increased DMI and milk yield over summer seasons. In contrast, Barnes et al. (2004), found no beneficial effects on heifers housed in warm conditions with dietary supplementation of 1.8 g/L Na2HCO3 and 3.5 g/L KCl. On the other hand, small but significant benefits using the same formulation were found in live weight and urine pH (indicative of improved regulation of acid-base balance) in cattle being transported by ship from Australia to the Middle East (Beatty et al., 2007). These cattle encountered periods of high temperature and humidity but were not clinically heat-stressed.

Potassium is the primary osmotic regulator of water secretion from cattle sweat glands, which increases the K+ requirements from 1.4 to 1.6% (West, 2002). Increasing the K+ content of the ration or in drinking water during the time that high ambient temperatures are expected may help. Supplementation with K+ and Na+ above recommended levels has delivered increased milk yield and/or DMI during heat stress in some studies (Mallonée et al., 1985; Schneider et al., 1986) but not others (Schneider et al., 1988, West et al., 1991; 1992). The meta-study performed by Sanchez et al. (1994) revealed increased DMI with increased dietary concentrations of P, Na+, and K+, with a very strong effect from increased Ca+2. Dietary Mg+2 was also effective till a certain concentration. These analyses are in part reflective of the complex dynamics of cation uptake in the gut. For example, any increase in dietary K+ will increase the competition for absorption of Na+ and Mg+2 in the intestine, so the inclusion rate of these cations in rations needs be adjusted upwards (West, 2002). High Cl- diets were consistently detrimental to improving DMI, and are likely to induce acidosis since absorption of Cl- which occurs in the ileum and colon requires a 1:1 exchange with HCO3- (West et al., 1991). Urine pH also decreases as renal excretion of Cl- is accompanied by excretion of an ammonium ion, NH4+ (West et al., 1991).

In optimising diets, nutritionists calculate the Dietary Cation to Anion Difference (DCAD) to characterise dietary electrolyte balance. In the simplest form of DCAD is the sum of the concentration of the major cations, Na+, and K+, minus the concentration of the major anion, Cl-. The units used are milliEquivalents (mEq)/100 g feed. The most efficacious range of mEq for production and production under heat stress has been the subject of many studies. Even under thermoneutral conditions, DCAD has a strong influence on feed intake and milk yield. Hu and Murphy (2004) performed by a meta-study of 17 trials conducted mostly in Holstein cows. They found a curvilinear (quadratic) relationship between DMI and DCAD whereby DMI improved up to a DCAD level of 40 mEq/100 g feed dry matter; similarly, a quadratic relationship was determined between milk yield and DCAD with the effect peaking at 40 mEq/100 g feed dry matter. West et al. (1991, 1992) also reported, that across varied DCAD diets consumed by lactating cows, a very strong positive influence of DCAD on DMI, blood HCO3-, pCO2 and pH, and a linear relationship with milk yield, regardless of ambient temperature. Cation source, Na+ or K+, had no affect (West et al., 1992). These researchers and others have found that the high mEq diets were beneficial during high heat load by ensuring adequate blood buffering capacity, regardless of the cation used or ratio of Na+ to K+ (e.g. Wildman et al., 2007). The addition of electrolytes would be best timed if added immediately before (if possible), during and immediately after a period of high ambient temperatures.

6.5 Feeding strategies

Management strategies to reduce or alleviate heat-stress-related production losses are warranted (Mader et al., 1999a; Mader et al., 2002). Reducing or alleviating the detrimental effect of heat stress on feedlot cattle is required for optimum performance during the summer months and to maintain the welfare of cattle. Strategies such as altering feeding time (Brosh et al., 1998), the amount of feed offered (Mader et al., 1999a; Davis et al., 2003) and manipulation of the dietary constituents (Gaughan et al., 1996; Mader et al., 1999a) have shown promise. An excellent review of nutritional strategies for managing heat-stressed dairy cows has been published (West, 1999), and there may be some application to feedlot beef. Dietary manipulation has been shown to be beneficial for reducing the effects of heat stress in beef and dairy cattle (Beede and Collier, 1986; Schneider et al., 1986; West et al., 1991; Mader et al., 1999b; Granzin and Gaughan, 2002).

Feed intake influences an animal's ability to cope with heat load (Brosh et al., 1994: Reinhardt and Brandt, 1994). Total heat production (within the animal) is largely dependent on feed intake (Purwanto et al., 1990; Gaughan et al., 1997). Reducing metabolisable energy (ME) intake through feed restriction or programmed feeding is known to improve feed efficiency in ruminants (Hicks et al., 1990; Murphy et al., 1994; Galyean, 1999), possibly by lowering maintenance energy expenditure and increasing diet digestibility (Murphy and Loerch, 1994; Sainz et al., 1995). Furthermore, restricting feed intake has also been shown to reduce increases in tympanic temperature when cattle are exposed to high heat load (Mader et al., 2002). Lower metabolic liver activity and liver mass in lambs, and lower resting metabolism in heifers, has been observed when diets were fed at maintenance levels in comparison with those offered feed ad libitum (Wester et al., 1995; Yambayamba et al., 1996). Improved efficiency and reduced visceral organ mass were observed in lambs fed high-energy diets at 85% vs. 100% of ad libitum (Fluharty and McClure, 1997). Restricting ME intake by diluting high concentrate diets with fibre may have the same effect as restricted feeding of a high-energy diet. Furthermore, Arias et al. (2011) clearly showed that high ME intake in the summer increase body temperature and low ME intakes in the winter decrease body temperature.

A reduction in energy intake in cattle is followed by a reduction in metabolic rate (Turner and Taylor, 1983; Brosh et al., 1994), which in turn lowers maintenance heat production. The digestion of roughage (digestion rate decreases with decreasing quality) is not as fast as that of grain-based diets and cattle cannot consume the same weight of roughage as grain. Therefore, heat production in cattle fed roughage is likely to be spread out over a longer time than in cattle fed a grain diet. However, there is considerable debate on this point.

A further effect of limited DMI is a change in the diurnal range in core body temperature via a lower minimum body temperature. Reduction of the heat increment of feeding by dietary manipulation may partially protect cattle from excessive heat load (Blackshaw and Blackshaw, 1994). During summer, cattle limit-fed in the evening are reported to have a better feed-to-gain ratio than those fed in the morning (Reinhardt and Brandt, 1994), although Gaughan et al. (1996) reported that there was little benefit to afternoon feeding when limited night time cooling occurred. Dietary manipulation, either via changing the time of feeding or by restricting access to feed, may result in a lower body temperature when cattle are exposed to high heat load (Gaughan et al., 1996).

Reinhardt and Brandt (1994) found that restricted feeding programs were particularly effective when cattle were fed in the late afternoon or evening vs. the morning. A bunk management regimen in which bunks are kept empty for four hours to six hours during the day may be used to prevent peak metabolic heat load occurring simultaneously with peak climatic heat load. Even though this forces cattle to eat in the evening, it does not appear to increase night-time body temperature.

The benefits of using restricted feeding programs under hot conditions have been reported by Mader et al. (2002) and Mader and Davis (2004). In restricted feeding studies, Mader et al. (1999a) housed feedlot steers under thermoneutral or hot environmental conditions. Steers were offered a 6% roughage finishing diet ad libitum (LRA), offered the same diet restricted to 85% to 90% of ad libitum DMI levels (LRR), or offered a 28% roughage diet ad libitum (HRA). Steers fed the HRA diet tended to have a lower respiratory rate and had a significantly lower body temperature under hot conditions than LRA and LRR steers. LRR fed steers had significantly lower body temperature than LRA steers (Figure 6.4).

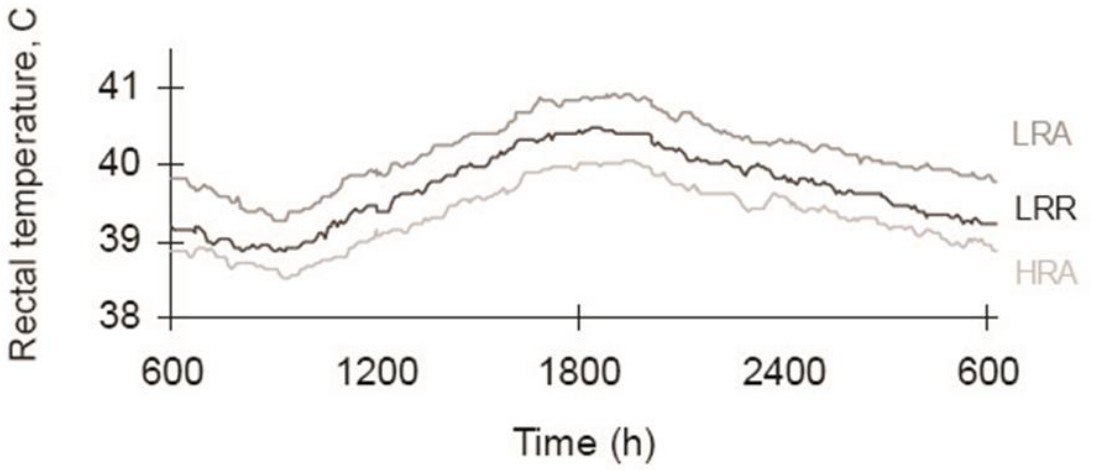

Figure 6.4. Effect of various diets and feeding strategies on the rectal temperature of steers exposed to hot conditions (LRA – low roughage diet, ad libitum; LRR = low roughage diet, restricted intake; HRA = high roughage diet, ad libitum (Mader et al., 1999a).

The lower body temperature of the HRA and LRR steers indicates that ME intake prior to exposure to excessive heat load influences the ability of cattle to cope with the challenge of hot environments and that lowering ME intake can lower core body temperature (Davis et al., 2003). Although slight feed intake reductions can occur with bunk management programs, particularly when they are first implemented, the benefits (lower core body temperature) of both bunk management and restricted-feeding programs are observed for several days after cattle are moved to a normal feeding program. Limit-feeding of high concentrate diets has been researched mainly as a management practice to obtain a specific target weight prior to finishing (Lawrence, 1998; Hicks et al., 1990). However, limit-feeding may be beneficial for alleviation of heat stress in feedlot cattle during the summer months. Davis et al. (2003) found that altering feeding time by keeping feed bunks empty during the hot portion of the day was nearly as effective as limit-feeding in reducing tympanic temperature, thereby minimizing the potential loss of gain associated with limit-feeding. Management strategies

designed to modify diurnal body temperature pattern could be useful for preventing vulnerability to heat stress and result in improved feedlot performance during the summer. However, the relationships between dietary management, ingredients and genotype are complex.

6.6 Feed additives

The use of dietary additives to reduce the impact of heat stress is an enticing proposition, especially as it would require only a small change to diet formulation. The potential uses of additives include:

1. a tactical response shortly before a heat event is expected to increase the resilience of cells to heat or the metabolic consequences of heat exposure,
2. a tactical response during a heat event to aid in the mechanisms of removing a heat load
3. a tactical response after heat exposure to aid the recovery of cells, tissues or metabolic pathways
4. a strategic response to include the additive(s) in the diet for a longer period of time as a risk management strategy to minimise the impact of heat stress on performance and welfare.

A key to the commercial success of using dietary additives is that the benefits must outweigh the additional costs, either direct benefits in animal performance (i.e. weight gain) or perceived or semi-quantified benefits to animal welfare. Of the many strategies investigated, a general conclusion is that the literature contains examples supporting the use of particular additives, but an almost equal number of cases where a lack of effect has been reported. This is perhaps not surprising when one considers the multiple responses invoked by exposure to heat. Furthermore, the extent of heat load and the capacity of animals to remove accumulated heat through other mechanisms (unrelated to the use of feed additives) are rarely consistent across studies and, in some cases, not fully reported. Therefore, in the following sections, we summarise the proposed mechanisms of action for a number of feed additives that have been investigated, and comment on the circumstances in which they are most likely to have a beneficial effect.

6.6.1 Monensin and glycerol

Baumgard et al. (2011) assessed the effectiveness of monensin in moderating the reduced milk yield of heat stress. The glucose appearance rate (Ra) was suppressed in both heat-stressed and pair-fed groups, and the addition of monensin into the diet caused a further 7-9% suppression of Ra. As might be anticipated, the monensin fed animals had 6-8% lower blood glucose levels. Glucose clearance as assessed by the glucose tolerance test was enhanced in both heat-stressed and pair-fed cows, and monensin improved the disposal rate. Plasma NEFA was markedly increased in monensin fed heat-stressed cows, overcoming the usually observed heat induced depression of plasma NEFA. Monensin also increased the NEFA response to adrenaline challenge in both heat-stressed and pair-fed cows (by 20 and 10% respectively). However, it did not re-direct glucose to lactogenesis thus did not improve milk yield in heat-stressed cows.

Glycerol has also been examined for its potential as an ameliorant of heat stress (Gaughan et al., 2009b). Glycerol, as a precursor for glyceraldehyde 3-phosphate, can be utilised by both the gluconeogenic and glycolytic pathways. It is also integral to the synthesis of triacylglycerols and phospholipids in the liver and adipose tissue. When added to the diet for growing feedlot Angus

steers, it did appear to reduce morning PS, and but did not improve any other physiological, biochemical or haematological parameters (Gaughan et al., 2009b). The glycerol treated steers also yielded higher $ value/carcase and greater dressed percentage, while reducing chilled meat hardness.

6.6.2 Betaine

Cronjé (2005) proposed that betaine (trimethylamine) may act as an ameliorant in heat stress based on its action as an organic osmolyte and thus assisting in maintenance of gut integrity. Osmolytes are compounds that affect osmosis across cell membranes. There are inorganic osmolytes (salts) and organic osmolytes, such as betaine. In heat-stressed mammals, water is lost from cells as it is needed for evaporative cooling from the skin (sweating) or panting. To counter this effect, cells increase the uptake of inorganic ions (i.e., K+, Na+ and Cl–) (Strange, 2004). However, these ions can denature and precipitate cellular proteins complexes. To avoid excessive damage, cells accumulate organic osmolytes (such as betaine) as these can control water movement across cell membranes without damaging cell structure or modifying cellular proteins (Moeckel et al., 2002; Burg and Ferreris, 2008).

Generally, mammals do not have a high need for osmolytes because the osmolality of blood is held remarkably constant. Nevertheless, certain cell types such as those in the kidney (renal medulla) do contain considerable concentrations of organic osmolytes. Importantly, other cells of the body must accumulate organic osmolytes consequent to being exposed to high concentrations of NaCl (or urea), such as what occurs during heat stress (Burg and Ferreris, 2008). This means that many tissues in the body, probably including the epithelial cells of the gut, synthesise or selectively increase uptake of organic osmolytes as a response to heat stress or dehydration.

Dietary betaine is known to influence hepatic cellular redox status and lipid metabolism. As a methyl donor, available betaine encourages the conversion of homocystiene thus sparing adenosyl methionine for phosphotidyl choline synthesis and triacylglyerol formation. Additionally, in the presence of high levels of betaine within the cell, glutathione is conserved, and along with reduced homocystiene, redox status is preserved and cellular stress reduced. Therefore, in heat stress betaine may have benefits over and above its role as an organic osmolyte. However, much of the dietary betaine is rapidly degraded by rumen microbiota, releasing acetate and trimethylamine which is likely to accelerate microbial growth. Dietary betaine has been observed in various ruminant trials to increase DMI and ADG, increase milk yield and milk fat, and increase ruminal VFAs; or do none of these. Wang, C. et al. (2010) also noted reduced plasma NEFA and hydroxybutyrate suggesting increased energy supply from the diet, and lowered rumenal pH.

There has been much recent effort in examining dietary betaine as a heat stress ameliorant with variable results. Gaughan et al. (2005) trialled a commercial preparation of betaine, Bos Koolus, in Angus steers that were fed a feedlot diet, housed in a climate controlled facility, and subjected to 5 days at 32°C and 66% RH. Supplemented steers maintained lower mean respiration rate and rectal temperature and higher DMI. A trial in the feedlot environment over a summer period where the rations delivered 20 g betaine/head/day, showed a slight increase in DMI, growth, exit live weight, and carcase and subcutaneous fat advantage, relative to un-supplemented steers. Interestingly, internal body temperature was not different between the two groups of animals. In a further trial,

betaine was added at various amounts in feed to growing feedlot Angus steers (with and without shade) over a summer season that saw 3 extreme heat events. Supplementation did not lend any advantage over shaded or unshaded controls in rectal temperature or internal body temperature, Panting Score, DMI or ADG (Gaughan et al., 2009b). Blood biochemistry, including plasma glucose was unaffected.

Most recently, a series of studies on betaine supplementation was conducted in various heat load experiments in sheep and dairy cattle (Dunshea et al., 2012; unpublished). Heat-stressed Holstein cows housed in a climate chamber maintained for 2 weeks at 39°C were supplemented at 35 and 75 g betaine/head/day. During heat load, betaine has no effect on milk production or characteristics despite decreased feed intake, which was associated with the dietary betaine and was additive to the heat stress induced reduction in feed intake. The betaine-supplemented cows also showed a slight reduction in water intake and respiration rate even with increased rectal temperature during heat load.

Sheep trials of dietary betaine as an ameliorant also returned a range of results which are difficult to interpret (Dunshea et al., 2012; unpublished). Sheep fed 2 g betaine/head/day during 3 weeks of heat load or thermoneutral conditions exhibited reduced respiration rate, water consumption and rectal temperature; while at the 4 g betaine/head/day dosage, these parameters were all increased. Plasma NEFA was lower, basal insulin higher, and glucose and lipids responses were altered to ACTH challenge in both betaine diets. At a higher rate of supplementation (35 g betaine/head/day) during an acute heat stress trial (4 days of high daytime temperatures), the betaine diet was similarly associated with increased body temperature, respiration rate and water intake, and reduced plasma NEFA and glucose. The conclusion from this research was that such high levels of supplementation only exacerbated the physiological and metabolic load on the sheep (Dunshea et al., 2012; unpublished).

The variable responses to betaine summarised here and reported in the literature may be based, in part, on some studies adding betaine in the diet at the same time that exposure to heat occurred, whilst, in other cases, betaine was fed for longer periods of time in advance of the animals being exposed to a heat event. In addition, the extent and duration of heat stress often differs between studies, so the effectiveness of providing an organic osmolyte such as betaine will depend on the other strategies that an animal can use to avoid accumulation of a heat load.

So, is betaine worth including in the ration of feedlot cattle at risk of being exposed to high temperatures? Most livestock feedstuffs are naturally low in betaine (Wang et al., 2004), although it is found in wheat. In intensive production systems based on grain and roughage that are naturally low in betaine, there may be value in providing betaine from an exogenous source as a risk management tool. The key questions, then, are how much of the betaine in the diet escapes microbial fermentation in the rumen and what does it cost to include it in the diet? Peterson et al. (2012) concluded that more research was required to determine the consequence of betaine on rumen fermentation characteristics and the optimal rate of supplementing dairy cows with betaine. Tomokazu et al. (2007) provided oral doses of betaine at 10, 25 and 50 g, and sampled digesta at the duodenum to determine if they could detect betaine post-ruminally. Betaine was detected at 0.19, 0.11 and 0.54 mg/ml, indicating that at least some of the orally-administered betaine can escape ruminal degradation.

In order for dietary betaine to be effective in protecting cells, its provision in rations prior to the heat event would be necessary since hyperthermia of itself will not trigger an increase in uptake of betaine by cells. Also the initial movement of water from cells into the extracellular spaces (hypotonicity) at the onset of heat exposure may actually reduce the transcription of the betaine transporters. Furthermore, if heat stress is severe enough to damage the epithelium of the gut (covered elsewhere in this review), the absorption of dietary osmolytes may well be compromised and may not be delivered to the cells where they are needed. The osmotic pressure around cells lining the gut (i.e. the hypertonicity) is actually highest when feed intake is high, so inclusion of betaine in the ration should occur before a heat wave when feed intake is high. Cronjé (2005) suggests that, because the timing heat waves are usually unpredictable, "the best strategy for protection of livestock would be to commence the addition of betaine to the feed with the onset of the warm season, and to continue until all danger of heat waves is past."

6.6.3 Chromium

Over 50 years ago, Chromium (Cr) was deemed an essential trace element in mammalian metabolism, and associated with glucose metabolism. Despite it being ubiquitous in diet and the environment, and its low daily requirement (33 µg/day for humans), it has become a popular dietary supplement to increase body lean mass and assist in control of blood glucose in diabetics (Vincent, 2010). It is now apparent that Cr is not an essential element and Cr deficiencies even under stress are rare in mammals, despite low uptake rates of 0.5-2% of ingested Cr (as a salt or complexed with small organic compounds). Nonetheless, in rat diabetes models and cell culture models, $CrCl_3$ and various forms of complexed Cr (e.g. Cr-methionine, Cr-picolinate) have been shown to alter insulin sensitivity and perturb both the insulin receptor and glucose receptors (Hua et al., 2012). Efficacy in clinical trials has not been as definitive. Supplemental Cr may have anti-inflammatory activity also. In vivo, four Cr^{+3} atoms are coordinated within a small 7-10 amino acid peptide which is occasionally termed chromomodulin (Chen et al., 2011). How the Cr-bearing peptide interacts with the insulin and glucose receptors remains unknown.

Dietary supplementation of Cr (in an organic form) has been investigated in several livestock species as an intervention during periods of stress or rapid growth. Overall, the metabolic and physiological effects have been variable (summarised in Anderson et al., 1997, Kegley et al., 2000), nevertheless, Cr supplementation of lactating dairy cows has consistently returned moderate increases in DMI and milk yield with no effect on milk quality (McNamara and Valdez, 2004).These changes apparently persist under heat stress or summer conditions, along with decreased plasma NEFA and/or cholesterol but plasma glucose was not affected (Al-Saiady et al., 2004; Soltan, 2009; Nikkah et al., 2011). Contrary to the beneficial effects in dairy cattle, growing beef cattle have not experienced improved DMI or ADG despite an apparent increase in insulin sensitivity (Kegley et al., 2000;). Dietary Cr supplementation of chronically heat-stressed growing sheep was also trialled (Dunshea et al., 2012 unpublished; Spears et al., 2012). Supplemented animals did have improved feed intake and very nearly maintained body weight comparable to thermoneutral controls. Intriguingly, the authors indicated a strong gender effect which they have yet to fully explain.

6.6.4 Hormone growth promotants

Various hormone growth promotants (HGPs) have also been assessed for their effects on the metabolic and endocrine responses of heat-stressed cattle. Little is known of the effect of HGPs on internal body temperature. The expectation is that estrogen based HGPs would increase internal body temperature through increased thyroid hormone secretion and thus increased metabolic activity (Kennedy and Cronjé, 2005). Androgen based HGPs should decrease feed intake, thus decreasing heat load from fermentation, and internal body temperature. Mader and Kreikemeier (2006) followed the influence of 5 different HGP treatments on the responses of feedlot heifers fed finishing diets over summer and winters conditions. The HGP treatments were estradiol-17 implant or trenbolone acetate (androgenic) implant, estradiol+trenbolone implant, or melengestrol acetate in feed or estradiol-17+trenbolone implant and melengestrol acetate in fed.

Over the summer growing season, mean tympanic temperature was not affected by any of the HGP treatments; however, maximum tympanic temperature was lower in the estradiol, melengestrol and estradiol-17/ trenbolone/melengestrol treated animals. The remaining treatments did not affect maximum tympanic temperature. Only the estradiol implant significantly increased minimum tympanic temperature, thus these animals were likely to be dissipating less heat overnight. Over summer, all HGPs increased plasma IGF-1 except melengestrol acetate relative to untreated controls. All treatments increased plasma T4 levels (especially melengestrol), but the picture is not so consistent for plasma T3. The estradiol-17+ trenbolone implant (with or without melengestrol) reduced plasma urea nitrogen.

6.6.5 Niacin

The potential benefits of added niacin in reducing the heat load of animals is entirely dependent on the outside temperature being lower than the core body temperature or that fans or sprinklers are available to remove the heat from the skin surface (Wrinkle et al., 2012). Niacin (vitamin B3, nicotinic acid) can indirectly invoke localised endocrine responses promoting vasodilatation. In humans this is known as a ‘flush’ response. Dietary niacin supplementation can result in activation of the niacin receptor on skin mast cells (Langerhan’s cells) with subsequent production and release of arachidonic acid from cell membranes and intracellular lipid stores. Arachidonic acid is the precursor for prostaglandin synthesis, and in the epithelial micro-environment, synthesis of prostaglandin D2 and E2 ensues. These potent molecules find receptors on local smooth muscle cells around small blood vessels allowing them to relax and thus increase local blood flow and heat loss from the skin (reviewed in Kamanna et al., 2009). Dietary niacin (or naturally-derived niacin from rumen microbes) is likely to only have noticeable effects when used to trigger a burst of heat loss, and thus aid in removal of a heat load under moderate heat stress. However, when ambient temperature is low, it is unlikely to be a useful strategy to dissipate heat (and avoid accumulation of a heat load) at times of peak heat production.

Early niacin supplementation trials in dairy cows observed improvement in milk yield, however, Di Costanzo et al. (1997) could not reproduce this beneficial effect despite a small decrease of rump skin temperature at high niacin dosage (36 g/d/cow). Given that most unprotected dietary niacin is quickly consumed by rumen microflora, Zimbelman et al. (2010) tested the efficacy of encapsulated dietary niacin on lactating Holstein cows in climate chambers under low humidity. There was enough

absorption of niacin in this experiment to increase plasma free niacin, and under thermoneutral conditions, niacin supplementation decreased rectal temperature. During heat stress, niacin fed cows recorded reduced vaginal temperature, and increased evaporative loss, so it is likely that the sweat glands activity of niacin treated heat-stressed cows was stimulated. The estimated average difference in stored heat in controls vs. niacin treated cows during heat stress was 182 kcal (Zimbelman et al., 2010) although this did not translate to improvements in feed intake or production (Zimbleman and Collier, 2011).

6.6.6 Antioxidants - selenium, Vitamin E, β-carotene, drugs

Dietary interventions to assist in maintaining a balanced redox status during heat stress in dairy cows have been attempted. Selenium is integral to the glutathione peroxidase complex which assists in removing free radicals from the cytoplasm. Calamari et al. (2011) supplemented the diets of lactating dairy cows over a summer season with 2 different sources of selenium (Se). Heat-stressed cows supplemented with high doses of Se recorded decreased plasma antioxidants, as well as (and interestingly) increased plasma NEFA alongside reduced plasma 3-hydroxybutyrate and urea. There was no effect of supplementation on milk yield. Those animals supplied with yeast derived Se responded to increased ambient temperature with reduced plasma TBARS, oxidants, oxygen derived metabolites and higher NEFA (relative to NaSe supplemented cows) (Calamari et al., 2011). Two independent studies on the use of dietary Se in heat stress sheep have been reported in 2012. Intravenous delivery of 5 mg NaSe three times over a 21 day period in hot (climate chamber) conditions was associated with reduced rectal temperature and reduced weight loss, even though respiration rate and DMI were not different from unsupplemented controls (Alhidary et al., 2012). In the second study, mildly heat-stressed sheep, maintained under climate chamber conditions, were fed a diet containing Se and Vitamin E at five-fold normal rations quantities. The supplemented animals showed reduced heart rate, and maintained feed intake and plasma antioxidant activity which fell in heat stress control animals (Dunshea et al., 2012; unpublished).

The effect of supplemental dietary β-carotene on milk yield and pregnancy rates in Holsteins in summer showed a minor improvement on milk yield only. Despite increased plasma β-carotene (1.5-2-fold over controls) and retinyl palmitate (but nor plasma retinol or Vitamin A) (Aréchiga et al., 1998).

The altered oxidative status of the gut epithelium as a consequence of hypoxia and/or acidosis has been nominated as one of the main culprits in damaging the cells and the tight junctions (Hall et al., 2001). Anti-oxidant drugs and proteins have been investigated for their ability to address redox imbalance in the gut during acute (and extreme) heat stress in anaesthetised rats (Hall et al., 2001; Lambert et al., 2002; Oliver et al., 2012). Intravenous delivery of aminoguanidine, allopurinol or a PEGylated form of superoxide dismutase was able to reduce radical production in the gut and may have improved cardiovascular function in this model (Hall et al., 2001). The simple amino acid, Arginine which is a critical substrate for the production of reactive nitrogen species, was ineffective (Hall et al., 2001).

Lambert et al. (2002) measured the gut permeability of anti-oxidant treated everted intestinal and colonic sacs excised from anaesthetised heat-stressed rats. None of the suite of small molecule anti-oxidants tested was effective in preserving gut integrity. In vitro treatment of rat tissues incubated

at 41.5°C and bathed with anti-oxidant drugs have not succeeded in reducing gut permeability with the exception of N-acetyl cysteine which also reduced protein carbonylation (Oliver et al., 2012).

6.6.7 Glutamine

Glutamine has also been expounded as a nutritional supplement to support gut mucosa during stress and disease. Glutamine is known to be utilised at high rates by intestinal cells for the production of energy, citrulline and proline (Windmueller and Spaeth, 1974, Mithieux, 2001). It has become a significant constituent in parenteral nutrition of severely ill patients. Singleton and Wischmeyer (2006) assessed 5 days of dietary supplementation with the alanyl-glutamine dipeptide in the feed of acutely heat-stressed rats. Prior to heat stress the intestines of the supplemented animals had increased expression of HSF-1, which was increased briefly after heat stress, along with increased expression of HSP70. Intestinal permeability was markedly improved and plasma endotoxin levels were lower 24 hours post heat stress relative to unsupplemented rats. Dietary supplementation with glutamine can be considered as a heat stress ameliorant and recovery candidate for feedlot cattle, with the caveat that while bovine jejunum intestinal cells do rapidly take up and metabolise glutamine, the rumen mucosa does not (Britton and Krehbiel, 1993).

6.6.8 New options worthy of (further) investigation

Recognition that inflammatory responses are likely to be an integral component of gut health and integrity during and after a heat stress event, has encouraged intervention with anti-inflammatory agents. Danapariod is a low molecular weight (~6 kDa) heparin-like molecule with anti-coagulant and anti-inflammatory activities, and is known to reduce inflammatory responses to LPS (Hagiwara et al., 2011). Intravenous administration of Danapariod to conscious acutely heat-stressed rats showed significant reductions serum IL-1, TNF and IL-6 levels, alongside less intestinal, liver and lung damage, reduced blood clotting activity and improved survival (Hagiwara et al., 2011).

The intrinsic biological activities of plasma and milk have been proposed as a means of reducing gut dysfunction due to weaning stress or gut infection. Diet supplementation of piglets with spray dried plasma (SDP) and plasma protein fraction (PPF; enriched for immunoglobulins) have been promoted as a possible replacements for antibiotics since supplemented animals appear to have improved growth rate and recovery from infection. For example, post-weaning piglets in their normal housing environment and supplemented with 6% SDP had less gut inflammation and immune involvement than their unsupplemented counterparts (Nofrarias et al., 2006). Similarly, Corl et al. (2007) found that neonatal piglets supplemented with 15% SDP vs. 15% soybean protein remained diarrhoea-free when subjected to a rotavirus challenge. They also had improved feed intake and better intestinal histology. Moretó and Pérez-Bosque (2009) summarised the evidence for modulation of immune, barrier and nutrient uptake function through supplementation with plasma protein preparations. Overall, in both pig and rat models, these supplements appear to reduce production of pro-inflammatory cytokines (TNF, INF and IL-6) and leukotrienes, and moderate the altered intestinal permeability. LPS is known to alter gut expression of glucose transporters and this effect is also countered by the plasma protein preparations. Apparently these products also moderate inducible nitrogen oxide synthetase activity in the inflamed gut, thus influencing oxidative status (Pérez-Bosque et al., 2010a,b). Supplementation of just weaned piglets improved transepithelial resistance, as well as, reduced expression of anti-inflammatory cytokines in both the ileum and colon (Peace et

al., 2011). To date, these dried serum supplements have not been tested in heat stress models of any kind.

Milk powders have been tested as ameliorants of gut dysfunction in heat stress models. Prosser et al. (2004) investigated bovine colostrum and goat milk powders in acutely heat-stressed rats. On taking internal body temperature to ~42°C, the rats experienced a 34-fold increase in intestinal permeability to 51Cr-EDTA. Bovine and goat milk powders, added to the diet at 17 g/kg feed, were able to reduce the movement of this tracer by 27 and 10%, respectively. Intense exercise is also known to bring on gut disturbance in part due to restriction of splanchnic blood flow. Male athletes were assessed after being subjected to intense exercise following 14 days of diet supplemented with 20 g bovine colostrum/day. Plasma GLP-1 was slightly reduced and intestinal permeability rose only 0.6-fold relative to the 2.4-fold experience by unsupplemented controls (Marchbank et al., 2011). Internal body temperature was unaffected by supplementation. These researchers looked to colon carcinoma cell line cultures to determine mechanisms whereby the milk constituents may be having an effect. On exposure to 39°C for 4 hours, the cells grown in colostrum supplemented medium were characterised by caspase activity and protein levels of Bcl-1 and Baxα (all markers of apoptosis /cell suicide) similar to the activity and protein expression levels of cells maintained in thermoneutral (37°C) conditions, and in contrast with cells from unsupplemented culture. Additionally, the transepithelial resistance of the cell monolayers was improved in the supplement culture cells after exposure to 39°C.

7 Current status of international and national research

Published research often lags behind current findings by 2-5 years, and of course much remains unpublished. To ensure currency of the review in heat stress nutrition for feedlot cattle, several overseas sites were visited to meet with international researchers who are actively conducting heat stress research in cattle (i.e. applied feedlot nutrition, heat stress physiology, nutrition and/or metabolism in cattle). The targeted overseas groups (and their facilities) were at the University of California, University of Arizona, University of Missouri, Meat Animal Research Center (MARC), Universita Degli Studi Della Tuscia and Wageningen University. The visits to these groups included assessment of their cattle research facilities. US feedlot nutrition consultants were also visited to access on-the-ground knowledge and progress in private sector research efforts. Much of the industry knowledge will be unpublished.

In preparation for our visits, we provided our overseas colleagues with some background to our visit, our area of interest and questions we would put to them. The document we supplied is attached as Appendix 8.3. The delegation comprised Dr John Gaughan (UQ), Dr Dean Revell (CSIRO), Dr Gene Wijffels (CSIRO) and Mr Des Rinehart (MLA). Below are reports of the visit to each site.

7.1 University of California (Davies)

At the Animal Science Department we met with experimental scientists, Dr Cassandra Tucker and her PhD student, Ms Jennifer Chan and as well as biological and production systems modellers, Professors Ermias Kebreab and Jim Fadel. The farm manager, Dan Sehnert, gave us an extensive tour of the university's livestock research facility near the campus.

Dr Cassandra Tucker conducted a 3 year post-doctoral term with AgResearch (Hamilton NZ) where she initiated her studies in cow comfort in thermal stress. She has continued with this line of enquiry on taking up her post as Assistant Professor (Animal Science) with UC Davis approximately 5 years ago. Much of her study has focussed on shade seeking behaviour. She presented data supporting

- Aggressive interaction between dairy cows with limited shade is temperature dependent, but interestingly aggression is higher in milder conditions, rather than under very hot conditions.
- If there is no shade, aggregation occurs at water troughs
- Shading has a big effect on body temperature trajectory

Ms Jennifer Chen, a 2nd year PhD student is supervised by Dr Tucker. Ms Chen presented her ongoing analyses of free choice sprinkler vs. shade vs. no cooling conducted in NZ. She has found that the very low heat loads in this experiment resulted in few correlates with ambient temperature. However, her recent Californian data exhibited less variability and is more predictive with increasing ambient temperature. Her new work is with steers cooled by 2.6 L/min sprinklers. She found this means of cooling mitigated depression of DMI (i.e. DMI was maintained). Her next experiment will examine rate and droplet size delivered by sprinklers, focusing on animal preferences and physiological responses.

Ms Chen prepared a table and presentation describing their use of commercially available temperature loggers (Table 8.1). In summary,

- iButtons – collect data for 1.5 days and can be placed anywhere on the body (can also be used within the body after wax coating is applied). Accuracy: 0.-1.0°C. They have been used for determining vasodilation – i.e. the temperature difference between two different locations on the body. (see Figure 7.1a)
- HOBO U12 – stainless steel – easily cleaned for reuse (approx. $AUD325 each) (see Figure 7.1b)

She noted that they have not yet discovered a logger with good accuracy (i.e. +/- 0.1 or 0.2°C) and long battery life. Most of their work used vaginal loggers since rectal devices (e.g. Figure 7.1c) may cause inflammation of the rectum within 10 days.

Professor Ermias Kebreab outlined a project proposal submitted to an internal competitive fund. The premise was to develop a heat balance model initially for beef cattle, and to extend the model to diary and use for shade and sprinklers. The model will include parameters such as heat load, body temperature, milk yield, DMI, respiration rate, sweat rate, etc.

Professor Jim Fadel is working on a heat exchange model initially published in J Animal Science (2011) (adapted from De Shazer et al., 2009). The aim of the model is to predict behaviours that will help with heat stress management. The model does not include conduction since this is most difficult input to measure, but does incorporate up to 24 parameters such as vasodilatation, respiration rate at two different time points, sweat rate, ambient temperature etc. It assumes no interaction between parameters. He mentioned that a pH balance model was published in J Animal Science (2011) by Adele Thompson, and a meta-analysis of thermal balance in *Bos indicus* and *Bos taurus* was published this year (2012). He foresees the evolution of models derived from iterations

of development, meta-analyses, and global sensitivity testing. He indicated that global sensitivity testing is useful if few parameters interact with each other.

Mr Sehnert distributed a handout detailing UC Davies extensive and various livestock facilities which accommodate beef cattle, dairy cattle, dairy goat, sheep, swine, horses, and a wide range of domesticated and wild avian species. There is also a meat processing plant, a rodent colony and aquaculture facility. Full information can be obtained from Mr Sehnert (djsehnert@ucdavies.edu).

The farm tour included an inspection of the climate chambers. The 2 climate controlled rooms were rudimentary in setup, having been fitted retrospectively, with very limited humidity control, constant temperature only (i.e. cannot be programmed to diurnal cycling) and limited capacity for large animals (4 animals) (Figure 7.2a). One room was used consistently to deliver the heat load and the other set to thermoneutral conditions which were measured and recorded by a simple wall mounted temperature gauge connected to a Hobo data logger (Figure 7.2b). The “Biobubbles” were set up by Associate Professor Frank Mitloehner, Cooperative Extension Specialist at UC Davies to measure emission from cattle in a pen situation. The Biobubbles are very robust and consisted of 3 polyethylene tarp covered semi-cylindrical housings, supplied with evaporative air-conditioning, and possibly some rudimentary humidity control (Figure 7.3). There is some solar radiation penetration. The Biobubbles are not currently in use.

The research feedlot has a total capacity of 750 head and was not currently in use (Figure 7.4). It consists of 28 group pens, a trial barn that contains 136 individual pens, and 8 additional pens for livestock air quality research. Feed intake and body weight measurements are collected manually, with no apparent setup for radio transmission of animal temperature or other data. Post-graduate students studying agricultural sciences would appear to be the main source of labour. One useful trick developed by a heat stress researcher was the use of strings of red lights (party lights) for night-time filming of animals as an alternative to a single large intense red light. The small lights do not influence animal behaviour or generate heat.

Figure 7.3 Climate controlled polytunnels at University of California (Davis). A. exterior aspect. B. Site for air-conditioning bank (currently not installed). C. Internal view .

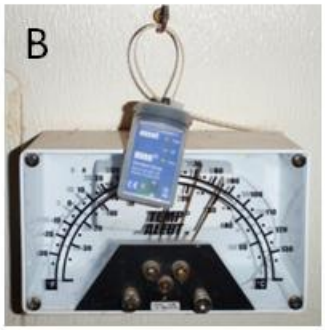

Figure 7.2 .Climate chamber (University of California, Davis) A. interior of one of the climate controlled rooms B. Wall mounted temperature and %RH monitor and logger.

8 Current Status of International and National Research

8.1 University of Arizona

The 'Collier' group at the Department of Animal Science was established by Professor Bob Collier and has been active in heat stress research since the mid 1980's. The current focus of the group was the study of environmental effects on gene expression in domestic animals. The research program utilizes both practical management models, as well as controlled environmental facilities with a focus on heat stress in dairy cattle. Environmental effects on gene expression are then evaluated using gene expression microarrays, real time PCR, and northern blots. Until very recently, the active researchers were Drs Lance Baumgard, Jessica Wheelock and Robert Rhoads. They have established

an elegant experimental design whereby heat-stressed cows in climate controlled conditions are matched with pair-fed thermoneutral controls. In this way, they have begun to disaggregate the effects of reduced feed intake from the effects of the added heat load.

Figure 7.4, The research feedlot at UCDavis

Professor Collier was not present when we visited at UArizona. We met with Professor John Smith who has been with UArizona for about a year as a Dairy Extension Specialist. Prior to coming to Tucson, he was a Dairy Science Extension Specialist at Kansas State University. His interests include cow comfort, heat stress, milking parlour performance and management of expanding dairies. John is clearly production focussed with an interest in heat stress. Dr Smith is well aware of time to recovery (of milk yield) after a heat stress event and is also concerned as to the long term deleterious effects of heat stress on young heifers and their future growth and milk productivity. He has worked with flip fans, sprays, and shade in various dairy set ups and is currently experimenting with cooling floors in commercial dairies.

We also met with Mr Laun Hall, a 2nd year PhD student supervised by Professor Collier. Mr Hall presented data obtained during a DAFF (Australia) funded project. Under chamber heat stress conditions, dairy cows fed 80 g/head/day betaine in their diet show normal glucose levels, similar to thermoneutral animals pair-fed on the same diet. The betaine treated animals have decreased water intake and increased rectal temperature. The thermoneutral high dose betaine cows may have shifted the excess N (from betaine) into milk protein production. The interaction between betaine and gut microbial population is unknown. These data was presented at American Society of Animal Science 2012 meeting (Hall et al., 2012). We also discussed some of the recent work on sheep and dietary supplementation with betaine (Dunshea et al., 2012; DAFF project). Plasma glucose was lower under heat stress vs. thermoneutral conditions, with the difference being the greatest with control diet; a modest difference with the 'mid'-dose dietary betaine, and only a small difference with the high dose betaine treatment. The data suggests that betaine may have the ability to reduce the hypoglycaemia effect of heat stress. The increased water intake associated with heat stress also reduced with addition of betaine to the diet. For control, mid and high betaine diets, water intake

was 108, 110, and 115 litres/head/day under thermoneutral conditions, compared with 140, 123 and 123 litres/head/day (respectively) under heat stress. These effects appear greater than the corresponding effect on feed intake, which was: 44-45 kg/day under thermoneutral conditions compared with 40, 38 and 38 kg/d for the control, mid and high betaine diets, respectively.

Mr Hall showed us around the facilities at the William J. Parker Agricultural Research Center. The laboratory space is not large but appropriately set up for cell culture, basic molecular biology techniques, and microscopy. The animal facility is well equipped with surgery, feed storage and preparation rooms; animal holding facilities (ideal for pig and small ruminant nutrition work); and climate chambers. The climate chambers comprised two independent rooms and an additional two rooms under construction for experiments with sheep. The climate facility was purpose built and is capable of delivering solar load; but this has not been used more than a couple of times (Figure 7.5a). Each of the two large animal rooms, houses six cows with capability to measure milk yield, and water and feed intake (Figures 7.5b and c). The rooms have excellent temperature, humidity, and light monitoring and control; are easy to clean, are equipped with 24 hour camera monitoring and automated recording; and adjacent viewing rooms (Figures 7.5d and e). Each pen is furnished with soft water filled mats to improve cow comfort (Figure 7.6a). Monitoring of individual animal rectal temperature and posture is conducted using a U12 Hobo probe and logger and Hobo pendants loggers (one each mounted on a foreleg and hind leg) respectively (Figure 7.6b and c).

The climate chambers are being modified to allow air sampling and analyses of gases (O_2, CO_2, CH_4). The maximum stay in the rooms has been 21 days. Two dedicated engineers are required when chamber experiments are running to ensure optimal operation of the considerable engineering infrastructure housed in the floors above the chambers (Figure 7.7). The internal charge rate for running the rooms is $25 for each room per day. The external use charge may run up to $150/day.

Figure 7.5 Interior aspects of the climate chambers at University of Arizona. A. Solar radiation installation. B. Pen arrangement. C. Milking line and low water flow drinkers. E. Temperature and humidity reader and logger.

Dr Benjamin Renquist has recently accepted an Assistant Professorship appointment with the department. His interests centre on metabolite signalling in appetence and satiety. Dr Renquist's interests include the physiological control of feed intake during stress. Stress can either induce or suppress feed intake. Heat stress suppresses food intake, while nutritional restriction increases food intake He proposes that β-hydroxybutyrate signalling through the GPRc109A acts as an appetite suppressant, and that this is the main cause for decreased feed intake in heat stress. Niacin can also bind this receptor at a lower affinity and thus could possibly compete with β-hydroxybutyrate. A second receptor, GPRc88 may also be part of the story. His research will focus on the metabolic changes that occur under both of these stressors to better understand how stress mediates changes in food consumption. At this stage, Dr Renquist will pursue rodent models using C57BL6 mice; heat stress in cattle is of secondary interest only.

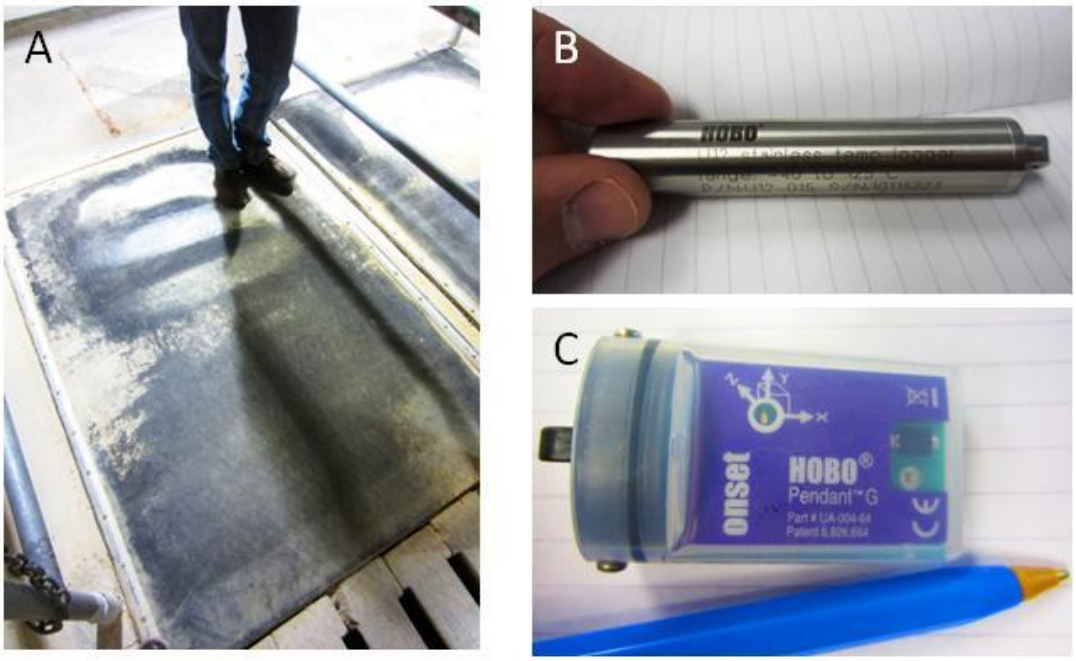

Figure 7.6. Animal comfort and monitoring within the climate rooms at the University of Arizona. A. Water filled rubber matting installed in each pen. B. U12 Hobo probe and logger for rectal temperature measurement. C. Hobo pendant monitor to recorded animal posture.

Figure 7.7. Engineering infrastructure supporting the operations of the University of Arizona purpose built climate chambers. A. Boiler system for waste processing and heat generation. B. Inflow and outflow air and liquid systems. C. Compressor units and controllers.

Niacin can also bind this receptor at a lower affinity and thus could possibly compete with β-hydroxybutyrate. A second receptor, GPRc88 may also be part of the story. His research will focus on the metabolic changes that occur under both of these stressors to better understand how stress mediates changes in food consumption. At this stage, Dr Renquist will pursue rodent models using C57BL6 mice; heat stress in cattle is of secondary interest only.

8.1.1 Succession planning at UArizona.

Professor Collier is likely to retire with the next 5 years. Drs Robert Rhoads and Lance Baumgard, both long standing members of the Collier team have moved on to new jobs and locations. Dr Baumgard, who was integral to the Collier group, has recently taken up a position as an Assistant Professor with the Department of Animal Science, Iowa State University. He is continuing his heat stress research but is working with pigs rather than ruminants. Dr Rhoads become an Assistant Professor in the Department of Animal & Poultry Sciences at Virginia Tech. This would appear to a teaching position. Mr Hill is the residual member of the team.

8.2 OT Feedyard & Research Center; XF Enterprises and Nutrition Services Associates, Hereford, Texas

We met with Mr Hollis Klett (President and owner of XF Enterprises), Mr Gary Holcomb, an on-staff nutritionist and industry consultant (NSA), and Dr Tanya Covey, Director of Research. NSA provides nutritional advice to commercial feedlots within the USA, Canada and Australia. In addition, Mr Klett owns and operates a 50,000 head commercial feedyard. A research feedlot that performs in-house and contract research for the industry Operates alongside the commercial feedlot.

We visited a 20,000 head feedlot (Kirkland Feedyard Inc) that employs Mr Holcomb as a nutrition consultant. The feedlot mills and mixes its own rations, as well as, growing corn to augment feed supply. It has an antiquated but quite sophisticated mill operation which uses the Comco Micro-Ingredient System to reproducibly and accurately add micro-nutrients into the feed mixing system (Figure 7.8; comco.wp.dev.id-3.net/solutions/micro-feeders/). Comco supplies the micro-ingredients (Vitamin E, Tylan®, niacin, others). Mr Holcomb is very much in favour of using this system and has advocated its introduction to Australia without success. The obstacle has been Comco itself, which sees Australia as too small and dispersed market for it to economically provide supporting services. Mr Holcomb mentioned that a Canadian company has developed and is distributing and installing a similar setup. They might be open to operating in Australia. Probiotics such as propionagens and lactobacilli are provided by the Nutrition Physiology Company (NPC; www.bovamine.com/about.html). New technologies for fine control of feed additives make it more feasible to use dietary additives. The equipment is usually part of the package deal with the manufacturer/distributor of the additive. However, Mr Holcomb suspects the additives sometimes are used because they 'can be' rather than clearly demonstrated cost-effective benefit.

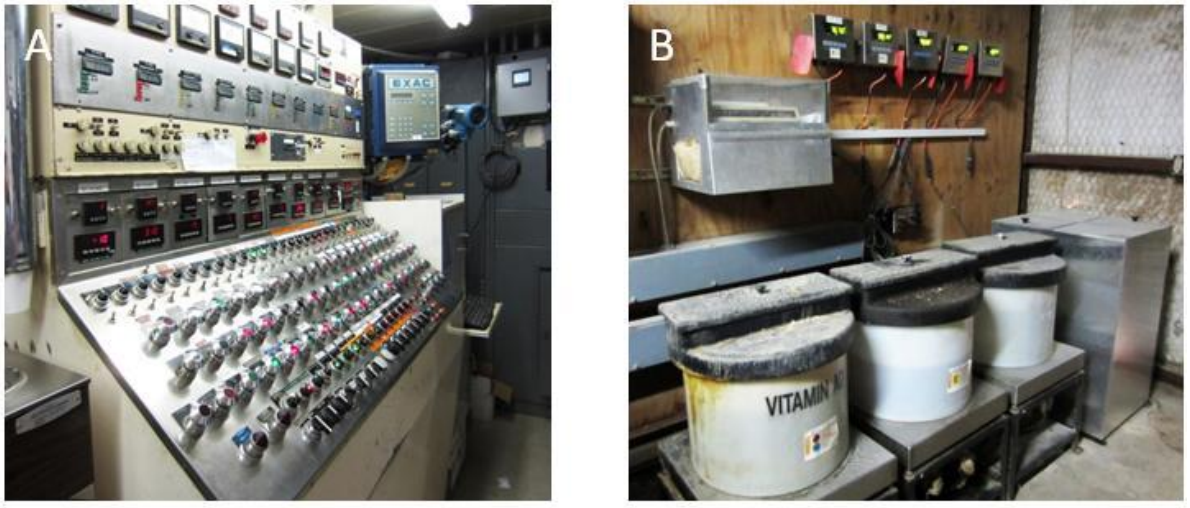

Figure 7.8. Components of the ration mixing system installed at the Kirkland Feedyard, Texas. A. . Feed mixer controller system. B. Bins of micro-ingredients supplying the Comco micro-feeder system.

We also visited D&J Dairy (5,500 head) and BC East Crowfoot feedlot (56,000 head) which runs a large number of Mexican cattle. Shade was only available at the dairy. Sprinklers are used at the dairy to assist with reducing heat load. Sprinklers at the feedlot are primarily used to reduce dust.

Shade is not considered to be necessary due to the *Bos indicus* content in the Mexican cattle, the generally strong winds, and sufficient night-time cooling. Last summer (2011) was characterised by high temperatures and poor quality roughage. While Mr Klett stated that they suffered no heat stress deaths, Dr Covey was confident that animals died from heat stress. Over summer, the 'Panhandle' area has consistent strong dry southerly breezes which assist enormously in ameliorating heat stress. Relative humidity which is a major stressor in many feedlotting areas is not a problem in northern Texas. Summertime sees the most weight gain since cold stress over the winter months slows growth.

In discussions with Dr Covey and Mr Klett, it was apparent that there is much controversy over the pen size used in trials for on-yard R&D. Large pen sizes are good if the variables can be controlled. The last trial conducted by OT used 20 pens of 80 animals. Dr Covey was conducting a trial investigating the cost of grain and productivity for a traditional vs. contemporary diet over 40 days. The company has recently constructed 14 new research pens; each pen can contain 80 – 100 head providing 150 ft^2/animal. The pens are 110 ft by 150 ft with 12'' of bunk space/animal. (The first in-house experiment to be conducted in the new pens will look at 2 different sources of β-agonist at 2 different doses.

Mr Holcomb outlined his nutritional interventions for the summer period. With heat stress, the ensuing rumen stasis has made critical that there is 'safe' food in the gut; i.e. roughage, not starch. For dairy cows, rations have increased K^+ plus dietary fat is adjusted to 3% from 1.5%. For beef cattle in summer, he alters rations to decrease starch and solubility, and uses good quality roughage to reduce energy. If rations have increased energy, again he will add good quality roughage, preferably, alfalfa. In his experience, alfalfa is the best summer hay to deal with reduced DMI. The rations also have increased K^+ and Vitamin B through yeast supplements. Wet distillers' grain is commonly used grain source with about 87% dry matter and seems to give a more consistent feed intake over the year. Niacin is also used throughout the year. In terms of heat stress research, Mr Holcomb's plea was to look into establishing and maintaining a positive acid-base balance and a proper balance of ADF:NDF during heat stress and follow-up period.

Other points of note were:

Current US veterinary costs are $14/head (compared with an estimate of $30-40 for British breeds in Australia; much less for Brahmans).

There is effectively a new industry around pre-conditioning cattle for feedlots; i.e. in between grass feeding and adapted fully 'on feed' in the feedlot. The aim is for all cattle to be medicated for health and adapted to the change in nutrition and conditions in the feedyard.

The productivity losses from BRD is decreasing but the cost of getting the decrease is increasing; long acting antibiotics cost $20/head

April (in the USA) is the worst month to transport cattle

8.3 UMissouri (Missouri)

This group at the Department of Animal Science is led by Professor Don Spiers (http://animalsciences.missouri.edu/faculty/spiers). The central theme of Dr Spiers' research has

been on heat stress problems associated with swine, dairy, and beef cattle production. He has used rodent models also. His projects over the last 10 years include studies of strategic cooling of dairy cows, development of models to define the thermal response of feedlot cattle to heat stress, and approaches to reduce the impact of fescue toxicosis. More recently, he has initiated studies to examine the genomics of heat stress in cattle with the goal of identifying markers of resistance and adaptation to environmental challenge.

We met with Dr Matt Waldron (a nutritionalist), Mrs Peggy-Anne Eichen, Dr Brad Scharf, Professor Monty Kerley and briefly, by Skype, Professor Spiers (who ironically was in Australia at the time). Discussions commenced with Dr Waldron who has recently joined UMissouri. He is interested in the cross-talk between the immune system and energy nutrition, and has focussed on peri-parturient immune-suppression in dairy cows. However, he has extended his interests to insulin resistance in heat stress and is working with insulin clamp models (presumably in rats). Dr Waldron pointed to immune involvement in heat stress as evidenced by increased somatic cell count (SSC)in milk, and high concentrate, low roughage feeds over summer causing subclinical ruminal acidosis (associated or exacerbated through high respiration rates contribution to respiratory acidosis) possibly invoking a systemic inflammatory response. Dr Waldron would be interested in pursuing gut inflammation due to heat stress and its connection to insulin resistance. His cell of choice is the neutrophil. Elvingen et al. (1992) saw decreased recruitment of neutrophils to mammary tissue in heat-stressed cows. The injection of an antigen elicits a reduced SCC in heat-stressed animals, indicative of a compromised immune system. However, Dr Waldron is basing his studies on peripheral blood neutrophils, rather than attempting to collect them from the mammary gland or from milk. The peripheral blood neutrophils from heat-stressed cows generate a lesser oxidative response than the same cells collected from thermoneutral cows. This reduced oxidative capability may in part be due to altered blood cortisol and glucocorticoids.

This group observed that heat stress during the dry period leads to less milk during later lactation. Also, if cows were cooled during the dry period, neutrophils during lactation have a greater capacity to release reactive oxygen (to kill bacteria) as measured by %oxidative burst. It seems that the metabolic challenge of parturition and onset of lactation increases the susceptibility of *previously* heat-stressed cattle. This raises a question about what other stressors might trigger this kind of response in previously heat-stressed cattle.

Dr Brad Scharf has just completed his PhD studies with UMissouri under Professor Spiers' supervision. Mr Scharf is focussing on better understanding the nexus between ambient conditions, animal thermal condition and productivity. Professor Spiers is particularly inspired with using the concept of hysteresis in any model they may develop or improve upon. They are working on an iPhone and android phone App which will be used as a heat stress prediction tool by lotfeeders. Currently it has only been developed for Missouri weather inputs, but will be incorporating weather data from across the USA and possibly Australia. There is a need for further development, and the Missouri group is currently looking at funding options. Mr Scharf has a post-doctoral position but will move from research to focus on the App development. More recently they have made a foray into responses by different genotypes to ambient conditions (Scharf et al., 2010). Mr Scharf presented data comparing Romosinuana and Angus breeds; comparing respirations rates, rectal temperatures and sweating rates (measured with a Delfin vapometer). Another interesting comparison was Florida-reared Angus vs. Missouri-reared Angus cattle. Data were also presented on the extra heat

load associated with cattle consuming endophyte-infected tall fescue, which raises an already elevated rectal temperature in cattle exposed to high ambient temperatures.

There was a general discussion around distinguishing between 'stress' and 'strain'. A stress might the same to all animals, but the strain/response it causes could be quite different between individuals. The Physiological stain index (PSI) (Moran et al., 1998 – military research with humans) is useful for acute exposure but doesn't really take into account adaptation (training). The relationship between ambient temperature and internal body temperature differs during the period of heat accumulation and the period of heat loss. A model of sensitive vs. insensitive animals is being considered where insensitive animals do not show the same rise in internal body temperature with an increase in ambient temperature. Both 'types' of animal show a diurnal fluctuation in internal body temperature as expected, with the insensitive animals generally having a lower internal body temperature throughout (i.e. both during the hot *and* cooler periods) although the difference was not evident on any one day.

Professor Monty Kerley is a nutritionist with a strong research focus on nutritional and metabolic influences on growth efficiency of cattle. He described the 3-5 day cyclical pattern in feed intake that is virtually always observed in *ad lib*-fed animals. He wondered how much of this could be related to heat load and dissipation. In addition is there a mechanism that links heat, energy metabolism and feed intake? During heat load, as during periods of energy expenditure/exercise, animals use ketogenic fuels rather than glucose. Soldiers consuming butterfat for breakfast had a lower body temp when relaxing, and a greater exercise capacity because of "better heat management".

Dr Scharf and Professor Kerley gave us a tour of their off-campus animal facilities, where several of the pens are equipped with telemetry and data collection set-up for GrowSafe and rumen bolus generated data (Figure 7.9). Their rumen bolus of choice is SmartStock Sensor which is a purpose designed and manufactured temperature data logger and sender (Figure 7.10). The sensor, once in the rumen settles in the reticulum in most animals (http://www.smartstock-usa.com/). The receiver box fitted with an omni-antenna will pick up intra-rumenal transmission from 300-500 feet away (~100 m). The recipient computer may be two miles from the receiver. The sensors are calibrated in a waterbath. They cost about $30 and can be set to a customised frequency if requested. GrowSafe appears to be a very robust system and very sensitive to individual variation in growth, feeding and drinking behaviours. Large amounts of real-time data are easily graphed but it still takes someone's time to trawls through such large data outputs. They make use of a Delfin Vapometer to measure sweat rate. This device costs around $6,000-7,000 and is not accurate under humid conditions.

Dr Scharf and Mrs Eichen showed us the comprehensive animal research facilities with the Animal Science Department buildings located on campus. The facilities provided for a wide range of animal research on a broad range of species, both livestock and non-domesticated. The climate chamber can house 8 cows and is furnished with milking lines to assess milk yield, and has good temperature and humidity control (Figure 7.11). The adjacent office allows monitoring of conditions and animals via computer relayed data from the chamber a viewing window. These facilities have been under-utilized in the last five or more years due to a lack of research funding and a move to modelling and application development.

8.4 MARC, Clay Centre, Hastings, Nebraska

This group which was established by Professor LeRoy Hahn in the 1980's, has a great depth of experience in measuring heat stress in feedlot cattle. Dr Tami Brown-Brandl is now leads the research effort using climate controlled rooms and a research feedlot (http://www.ars.usda.gov/pandp/people/people.htm?personid=691). Some information on their activities can be found at http://www.ars.usda.gov/SP2UserFiles/Place/54380000/Environmental_Management_Research.pdf. The ARS is the research arm of the USDA, with 2,000 scientists and a total of 8,000 employees who engage in all areas of agricultural research. MARC is the largest site of the ARS, covering research in genetics, meat safety, nutrition, reproduction, animal health and environmental management in beef cattle, swine and sheep farming. The site homes 110 federal government employees whose activities can be divided up as 50% beef cattle, 30% swine and 20% sheep research. The site is also home to 105 UNebraska staff who run the farm operation for teaching and extension purposes. The land under use is 13,800 ha; 9,700 ha for grazing; 1,200 ha for corn and 800 ha for alfalfa. The animal capacity is 7,000 cows; 3,000 ewes, 500 sows (farrow-to-finish) and a 6,000 head capacity beef feedlot.

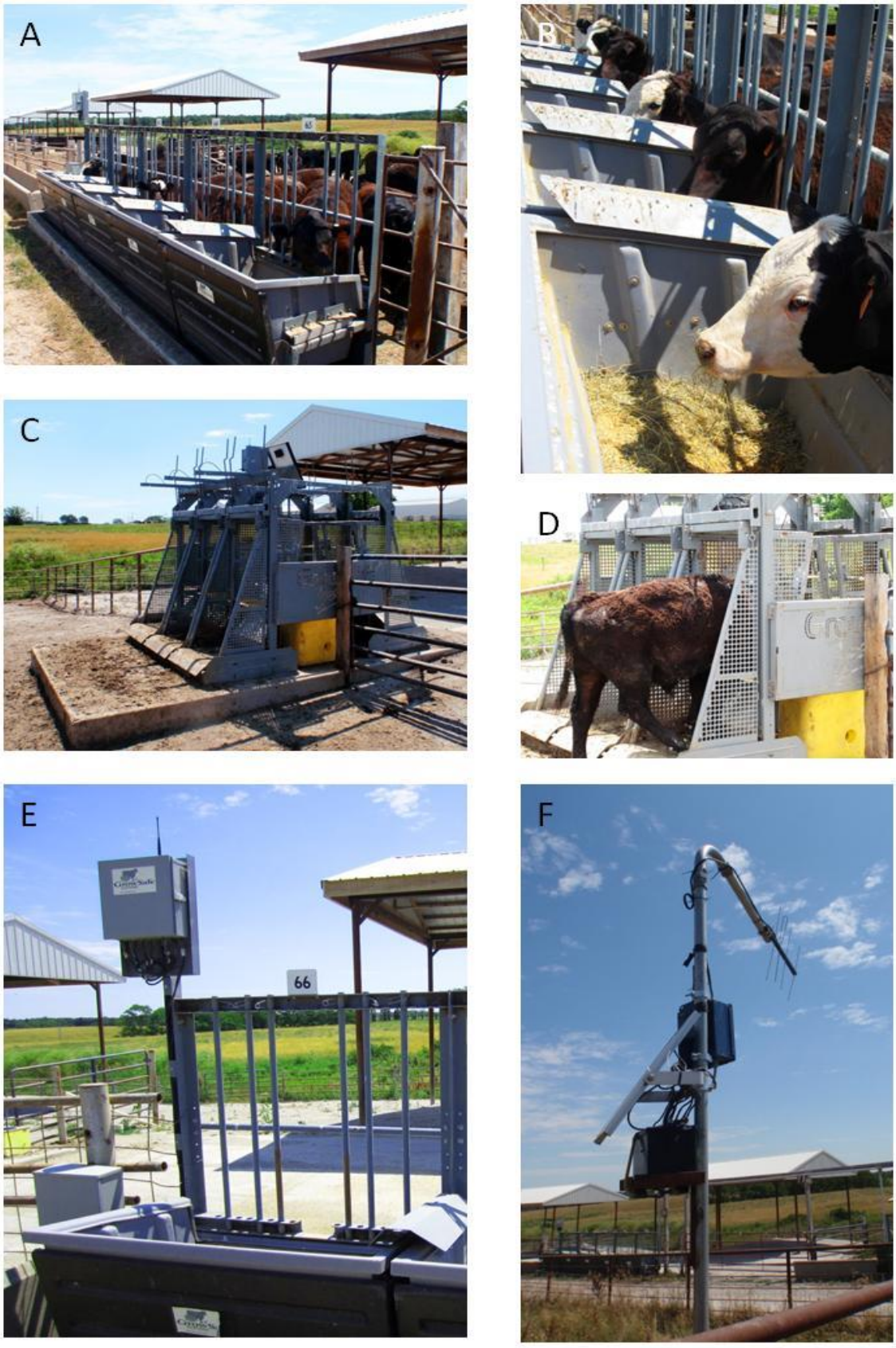

Figure 6.9 Growsafe installation in the research pens at the livestock facilities of the University of Missouri. A and B. feed-intake bins. C and D. water-intake and body weight measurement. E. Data collection and transmission box. F . Antenna to receive, amplify and transmit data to a lab computer 500 m distant.

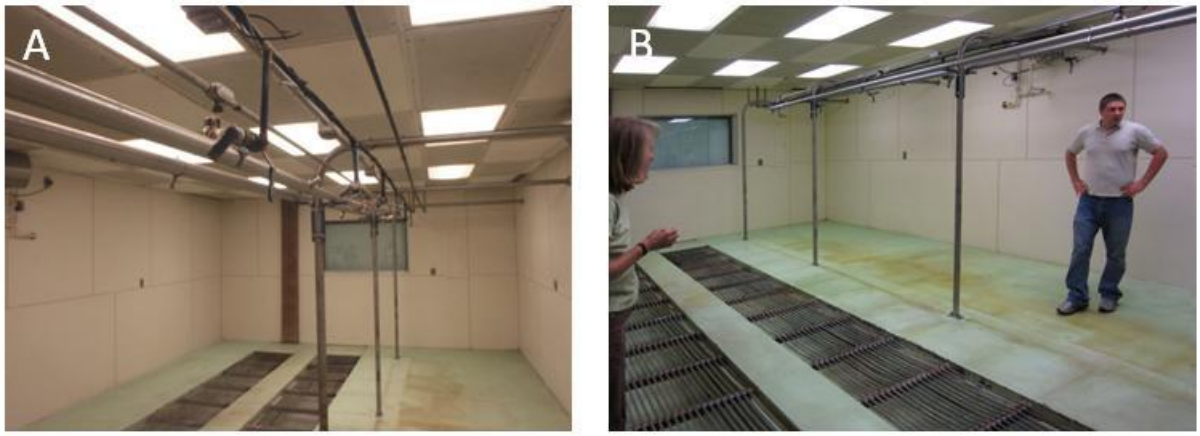

Figure 7.11. Interior of the climate chamber, Department of Animal Science, University of Missouri, Missouri.

We met with Drs Tami Brown-Brandl and Roger Eigenberg (both agricultural engineers), Dr Kristen Hales (nutritionist), Dr Carol Chitko-McKown (an immunologist), Dr Morgan Hayes (a new postdoctoral scientist, with Dr Brown-Brandl) and a PhD student (with Dr Chitko-McKown). The scientists presented their current work as summarised below.

Dr Brown-Brandl's current interest relates to heat stress and (i) animal susceptibility, (ii) environmental management and (iii) nutrition. Her data showed the relationships between ambient temperature and respiration rate or rectal temperature, highlighting differences between individuals, and how fitted regression lines can reveal sensitive and insensitive groups of animals. Differences between groups in the regression fitted to ambient temperature vs. respiration rate denoted

- lower regression line for light-coated animals vs. dark coated animals;
- higher regression line for pneumonia-treated animals than controls;
- more variable relationship with excitable vs. calm animals.

Other experiments with subsequent careful data analyses have shown that

- Cattle cooled with water when going through the chute at about 10 am showed lower internal body temperature around midday, but similar internal body temperature as unwetted animals around mid-afternoon. Most interestingly, the morning cooled cattle had lower internal body temperature at about 6 pm compared with the non-cooled controls. Early cooling might have important benefits to the loss of accumulated body heat in the evenings.
- Animals with markedly lower growth rates than pen-mates show a marked increase in growth rate when the heaviest animals were removed from the pen. This demonstrated that the initial low growth rate was not reflecting poorer genetics, but a behaviour-related response to access to the feeder.
- "time spent at a feeder' was a good predictor of pneumonia, with a decline being evident about 3 days before veterinary intervention was called for.
- Brown-Brandl has two major projects in progress

- Updating the housing and engineering standards for swine as the original work with completed in the 1980's. Since then both genotypes and diets have changed considerably.
- Development of a nationwide heat stress prediction tool which will be mounted on NOAA's National Weather Service site (http://weather.gov/). This tool predicts a respiration rate that then can be compared to the various categories of heat stress, thus informing the managers of likely heat stress load in coming days. The model/algorithm was developed from the 2011 shade and unshaded experiments.

In respect to recent heat wave losses, Brown-Brandl was able to tell us that the South Dakota 2007 summer saw >2000 feedlot head lost (unofficially, >7000 head); Nebraska 2009, 4,000 deaths recorded; and Nebraska 2011, > 6000 head lost.

Dr Roger Eigenberg has been working on improved shades for feedlots and shade requirements. He has compared utility of 0, 30, 60 and 100% shade cover (density of shade, not coverage of shade within the pen). Respiration rate for each group was in the order: 0 > 30 > 100 > 60. Interestingly, those in 60% shade had a lower respiration rate compared to all others, including those with 100% shade.

Dr Kristin Hales is an applied feedlot nutritionist and a relatively new appointment to MARC (started in March 2011). Her mission is to increase feed/production efficiency. CurrentlyDr Hales has several experiments running.

- The efficacy of Ractopamine (β-agonist) in well pedigreed animals. She is part of a multi-disciplinary team of molecular biologists, geneticists, physiologists and nutritionists. An aside: β-agonists upregulate expression of *E. coli* virulence factors.
- The role of fibre in feed byproducts especially from distillers' grain which contains about three-fold the fibre of corn flakes. The %roughage of a typical finishing diet is 7-9% depending on season. Kristin has a paper in JAS on nutrition from distiller's grain diets (Effects of corn processing method and dietary inclusion of wet distiller's grains with solubles on energy metabolism, carbon-nitrogen balance, and methane emissions of cattle: K.E. Hales, N.A. Cole, and J.C. MacDonald. J Anim Sci 2011-4441; published ahead of print May 14, 2012, doi:10.2527/jas.2011-4441).
- Nutrient balance study.
- Protein needs for different μ-calpain and myostatin genotypes

A tour of their animal facilities and equipment revealed four climate chambers, each chambers accommodating a maximum of nine steers. There has not been much work in the chambers over the last few years, and the chambers have not been maintained in a working condition. The rooms can be programmed to replicate real heat stress events. Four indirect calorimeters which can be used in the chambers measure ambient temperature and %RH and on-line O_2, CO_2 and CH_4. The setup requires the animal place its head through a sleeve into the respiration chamber from where air is sampled for gas analysis both for the on-line system and/or sample bags (Figure 7.12). The O_2 analyser is affected by atmospheric pressure.

Advice on ancillary equipment include

- Respiration monitors can collect up to 5 days of continuous data.

- Body temperature telemetry (the closer the logger is to the sensor the better the data).
- Vaginal temperature detector inserted into fingered rubber tube; different sizes for heifers and cows. They are reusable.
- Time lapse camera: Originally Birdcam from WingScapes. $200. Very weather resistant and very sensitive (Figure 7.13).
- Water flow meters – use an Omega meter which is very sensitive for low volume flow.

The feedlot complex comprises several large pens and numerous small pens with and without shade. Feed and water intake is measured using the Insentec feed system (the Dutch equivalent to Growsafe; http://www.insentec.eu/en/cattle-management/conventional/feed-station) which has the advantage of being able to record data from more animals per feeding bay (Figure 6.14).

We have since heard that Dr Brown-Brandl has been encouraged to focus on methane mitigation. Also Dr Roger Eigenberg will retire within the next 18 months. This leaves Dr Kristin Hales, a nutritionist interested in the application of genetics, as a potential collaborator at Clay Centre.

Figure 7.12. Indirect calorimeters housed in the climate chamber at the MARC, Clay Centre, Nebraska. A and B. Indirect calorimeters. C. Head sleeve. D. Temperature and %RH measurement. E. Low flow water feeder installed in the calorimeter.

8.5 Universita Degli Studi Della Tuscia, Viterbo, Italy

This is a very good and solid group led by Professors Nicola Lacetera and Umberto Bernabucci. They have been addressing heat stress in dairy cattle from a wide range of approaches, including cell biology, functional genomics, and rumen function and digestibility.

We met with Professors Lacetera and Bernabucci, and post-doctoral fellow, Dr Andrea Vitali. The other members of the department that have worked in heat stress are Dr Patricia

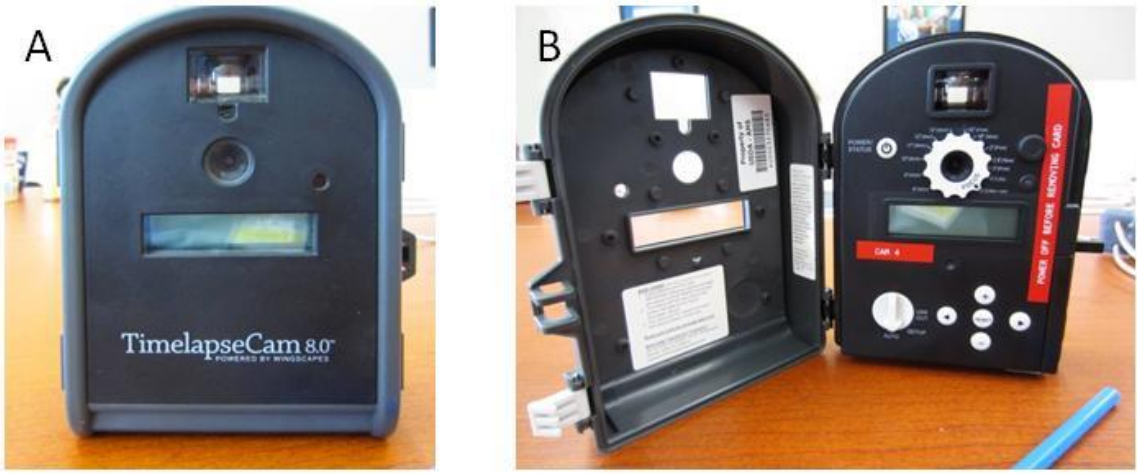

Figure 7.13. Time lapse camera (Birdcam from WingScapes) used by Drs Brown-Brandl and Eigenberg to monitor penned cattle. A. Exterior face. B. Internal components.

Figure 7.14. Insentec feed system installed in the research pens at the MARC, Clay Centre, Nebraska. A. External face of the feed bins. B. Controllers and data logger. C. Pen-side view of the feed bins. D. Internal view of a feed bin reveals the weighing system and ear tag readers.

Morera, Professor Alessandro Nadone, (retired, and living in Rome but very active) and Professor Bruno Ronchi (now very much occupied with administration and national university administrative politics).

Professor Lacetera introduced us to the group's projects. The group has broad interests in the metabolic, immune and reproductive effects of heat stress and have conducted field, chamber, and *in vitro* studies, as well as analyses of historical clinical data (see below). Their ruminant *in vivo* model contrasts spring and summer transition dairy cows, measuring growth (heifers), reproductive capacity, milk yield and quality (for cheese production – proteins (casein and α-lactoglobulin), fatty acids and lactose). They have conducted parallel immune function, metabolism, and oxidative stress studies in spring and summer transition dairy cows. With respect to milk quality, summer milk is characterised by decreased short and medium chain fatty acids, and relative increase in long chain fatty acids. It is unknown if this is due to dietary changes, (hay to grazing) or heat stress or a combination of both.

Recent studies include

- Analyses of retrospective data collected on dairy mortality by the government for general health and BSE monitoring. From the six years of data (300,000 deaths), the highest mortality was during 2003 which experienced a record hot summer. There appears to be a two phase relationship, with rate of mortality accelerating at THI >80. (Vitali, A., Segnalini, M., Bertocchi, L., Bernabucci, U., Nardone, A. and N. Lacetera. 2009 Seasonal pattern of mortality and relationships between mortality and temperature-humidity index in dairy cows. J Dairy Sci 92, 3781-3790.)
- Seasonal variation of milk quality and SSC in northern Italy (Po Valley).

New studies include

- Milk for a Parma-like cheese, Regano has a very specific problem in summer cheese production. There appears to be a seasonal link between *Clostridium butyricum* infection and growth in Regano cheese production (which spoils the cheese and milk). They are looking at *C. butyricum* in the gut over the summer season. In a related project, Umberto is looking at control of barn microclimate conditions to reduce *C. butyricum* infection of milk.
- A project proposal around adipokine regulation and expression, and altered glucose and lipid metabolism has just passed the first hurdle in a national funding round.
- HSP70.1 polymorphism and association of heat stress tolerance in dairy cows; both in vivo and in vitro assessments.
- Heat stress in transition goats and dairy buffalo cows
- Dr Morera is working with heat stress in rabbits (as a production species) and metabolic enzymes in a mouse heat stress model.

The group has run chamber experiments in years past (Figure 7.15), but expense and lack of enthusiastic young scientists limits the likelihood of future chamber work.

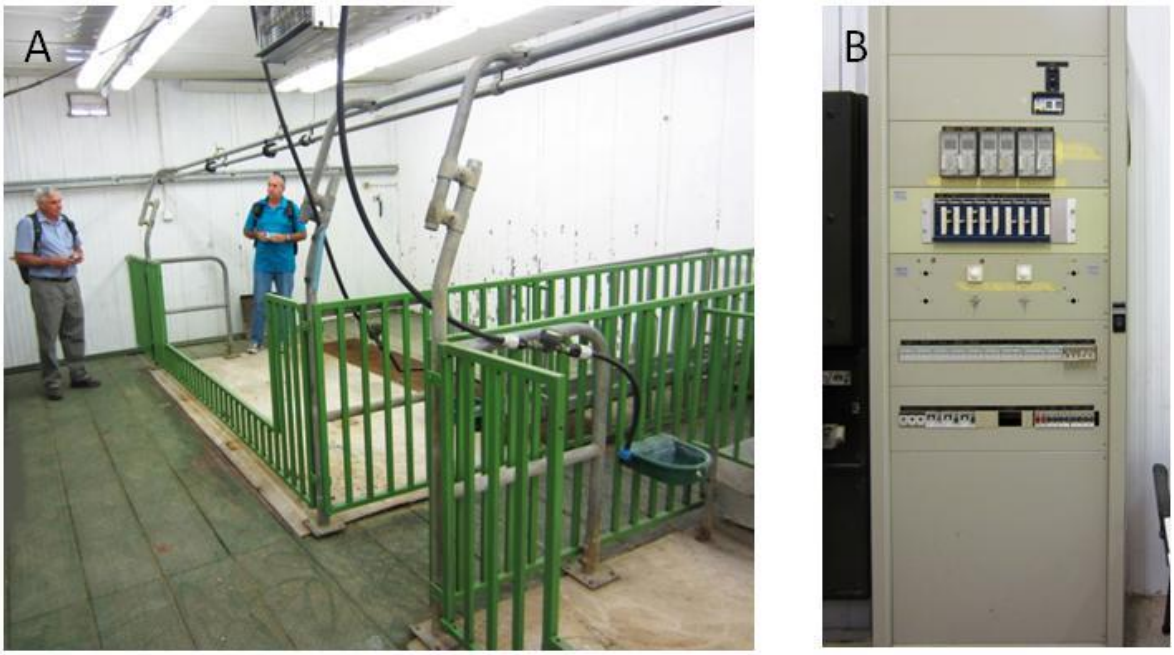

Figure 7.15. The climate chamber at theUniversita Degli Studi Della Tuscia, Viterbo, Italy . A. Internal view. B. Controller unit.

8.5.1 Immune responses in heat stress

In transition cows, the intensity of lipid mobilisation (i.e. high blood NEFA) leads to modification of immune cells in terms of their capacity to respond to stimuli (i.e. immune suppression). These findings are published in a series of papers from 2005. Palmitic and stearic acids have especially negative effects on immune cells. It is not clear how this relates to heat-stressed cattle given the fact that heat-stressed cows do not necessarily have elevated NEFA concentration despite being hypoglycaemic. The findings are based on an *in vitro* model using freshly harvested peripheral blood cells. NEFA mobilisation is associated with increased immune suppression. What is the relationship? Using their in vitro system, they have shown that addition of a plasma-like NEFAs mixture to culture media has an immune-suppressive effect on lymphocytes. *In vitro* studies of PBLs from heat-stressed Brown Swiss cows vs. heat-stressed Holsteins cows, found that the cells from Brown Swiss cows are less tolerant to chronic heat exposure than those from Holstein cows, and that the lower tolerance is associated with higher expression of HSP72 (Lacetera et al., 2006). Curiously, this conclusion is contradictory to anecdotal observations in Australia in that the Brown Swiss cows are doing better in warm conditions. The ability of transition cows to respond to LPS (as measured by weekly assays) shows a great range of responses between animals, but cows do rank consistently as high or low responders.

8.5.2 Metabolic acclimation to heat stress as seen in dairy cows, mice and from in vitro data

Relative to pair-fed animals, heat-stressed animals have reduced plasma glucose, cholesterol, NEFA, and reduced hepatic enzymes but increased plasma bilirubin (Ronchi et al., 1999). Increased basal insulin levels and increased glucose clearance is also characteristic of heat-stressed dairy cows and

beef cattle. Heat stress causes increased glucose uptake and utilisation by muscle and adipose tissue.

In transition dairy cows, the lipoprotein, ApoB100 is down regulated in periparturient period and early lactation. There appears to be a relationship between altered ApoB100 levels, lipid mobilisation and heat stress. In the record warm summer of 2003, hepatic expression of ApoB100 at both mRNA and protein levels was down regulated, along with plasma ApoB100, and negatively correlated with plasma NEFA and lipid accumulation in the liver (Basiricò L., Morera P., Lacetera N., Ronchi B., Nardone A., Bernabucci U. 2011 Down-regulation of hepatic ApoB100 expression during hot season in transition dairy cows. Livestock Sci 137, 49-57).

Professor Bernabucci's most recent research has investigated adipokines and HSP expression in heat stress. Using 3T3-L1 adipocytes cultured at 37 through to 42°C, for 24 h, he showed peak expression for adiponectin mRNA at 39°C and leptin at 41°C (where the cells must be in extreme stress). Secretion of these cytokines into the culture medium gave a similar profile. (Bernabucci, U., Basiricò, L., Morera, P., Lacetera, N., Ronchi B. and A. Nardonne. 2009 Heat shock modulates adipokines expression in 3T3-L1 adipocytes. J Mol Endocrinol 42: 139–147). The increased expression of these adipokines was not sustained after removal from heat stress (unpublished).

They have now extended their observations to their mouse model, comparing leptin, adiponectin, blood glucose and lipids of mice maintained at normal housing temperature (24°C), 35°C and pair-fed mice at 24°C. Interestingly, glucose and NEFA levels were the same in pair-fed and heat-stressed mice, but leptin, adiponectin and insulin levels were all increased in the heat-stressed animals. The liver samples from this experiment are being analysed by microarray by Juan Loor (Animal Science Department, UIllinois, Urbana).

In pursuing the heat shock protein story in these mice, they have found while the pair-fed mice had increased expression of HSPA1 (HSP70.1) in adipose, muscle and liver, but that heat-stressed mice had higher expression yet again. There was also increased receptor expression in all tissues although some interesting variations in tissue expression patterns were observed (Morera et al., 2012) will verify these findings in mice by repeating the experiment contrasting 29°C with 35°C. (29°C is considered thermoneutral for mice).

8.5.3 Oxidative stress

Heat-stressed transition dairy cows experience increased oxidative stress. Professor Bernabucci's research indicated a negative correlation for ApoB100 in the plasma and liver and plasma and rbc ROS. In a preliminary study in transition dairy cows, they have supplemented five cows with dietary selenium and Vitamin E for 25 day prior to calving. Relative to controls, these cows had increased plasma ApoB100 and cholesterol throughout transition, as well as increased milk yield and PCVs.

This group does well at maintaining a diverse animal and laboratory based research portfolio to sustain funding. They are enthusiastic about heat stress biology and possible collaborations with Australian researchers.

8.6 Wageningen University, Netherlands

Wageningen University's Department of Animal Sciences is renowned for its livestock nutrition and metabolism expertise. We met with Associate Professors Walter Gerrits and Jan Dijkstra. Professor Gerrits's expertise in pig and calf nutrition, intestinal physiology, energy and protein metabolism of farmed animals, and use of stable isotope in metabolism research. Jan's expertise lies with metabolic processes in farmed animals, particularly ruminants.

Professor Gerrits summarised the Animal Nutrition group's research activities. Currently they are working more with pigs than calves, with several projects in progress, mostly through 20-30 PhD students. The various pig projects are

- digestibility, use of canola (by $^{\text{in vitro}}$ studies, validation in poultry and finally pigs)
- the phosphate in diets is too high and soils are now saturated with it, therefore there is an interest in using phytases
- starch utilisation and optimisation of protein:energy interactions
- looking at rapid, moderate and slow fermentable starches
- some pig diets have up to 12-16% fat (which pigs can deal with)
- feeding by-products
- decrease grains
- increase wet by-products with matched concentrates often in liquid form. Often tricky problems with solubilities and seasonal differences
- using some dried distillers' grain but these are known to have heat damage protein and oxidised amino acids (lysine, methionine, cysteine)
- beer production by-products are also being assessed
- canola meal – high in phosphorus, and high fermentability.

The calf (veal) research program is moving away from milk replacers to increasing solid feeding to ensure development of the rumen. There are 3-4 PhD students on this program.

The laboratory houses good histology and microscopy suites. Experimental systems include *in vivo* stable isotope tracer studies (using an isotope ratio mass spectrometer), gas production analyses, and intestinal and fermentation simulations (with auto sampling).

8.6.1 Metabolism research

New experiments are being funded by the Agriculture Ministry and the various dairy feed industry organisations and companies. The EU aim is to reduce methane emissions by 5%/cow and supposedly 30% per L milk. Trials will be conducted in the newly built metabolism rooms looking at baseline methane emissions and emissions following nutrition interventions. Candidate nutrients/additives have been assessed in the *in vitro* digestion system, and often, those that rank highly disappoint during *in vivo* trials. Current trials underway will assess 4 different grass qualities at different N content. In preparation for the trials, the cows will have 14 days adaptation, and 4 days in the chamber. Feed intake will be controlled to eliminate this variable. Adaptation to increased dietary nitrite takes place over 4 weeks, altering the ratio between the old:new feed at a rate of 25% each week. With nitrite adaptation, methane production is reduced by 2%.

8.6.2 Other notes

Fatty acid profile of milk to improve human health – FrieslandCampina was advertising milk with decreased saturated fatty acids. Wageninen has had several students looking at fatty acid synthesis in the udder to decrease saturated fatty acids; and biodehydrogenation of fatty acids in the rumen.

Rumen acidosis – occurs 2 weeks prior to calving and can persist after calving. The group is interested in following expression of genes involved in VFAs absorption in the rumen and are also using stable isotope uptake analyses.

8.6.3 Facilities

A new building, housing the metabolism rooms has just been made ready for use. The construction was part of the consolidation of the Animal Science facilities to a single site. The newly designed metabolic/calorimetric rooms are impressive. The rooms are engineered for flexible rooming arrangements to accommodate cows, pigs, sheep, chickens etc. Each separate chamber can be controlled for air quality and flow, temperature and humidity conditions and for sampling regimes (Figure 7.16). Urines and faeces can be collected. Gas analyses are on-line and automated. The first experiment, a methane emission study, began in mid-2012.

Figure 7.16. Recently installed and commissioned metabolic chambers at the Wageningen University, Department of Animal Sciences. A. External doors into the individual chambers. B. Internal view of an individual chamber. C. Compressed air lines supplying the internal door sealant system. D. Automated on-line gas chromatographs and controllers units supporting the chamber operation. E. Supporting pumps, lines and valves above each individual chamber. F. Urine and faeces collection containers below the chambers. G. In-flow and out-flow gas and air lines leading to and from the individual chambers.

There are two sets of four rooms but they can only house seven dairy cows in one run. Professor Gerrits could not give the running costs since the setup is so new. Furthermore, the facility is currently 'owned' by the university and the department is 'leasing' it from them. In time they are

expected to pay for the facility. The actual management of the facility is with the department. Currently the facility is booked for experimental work till 2014. Professor Gerrits is keen to set up collaborations to ensure the facility is used after this time. He is not looking for full cost recovery.

8.7 Australian Facilities

8.7.1 Centre for Advanced Animal Sciences, University of Queensland, Gatton

The Centre for Advanced Animal Science (CAAS) facility was commissioned in 2010, and is a joint venture between The University of Queensland and the Queensland Government.

There are four climate rooms in which the temperature can be set to range from 8 to 42°C; and relative humidity from approximately 25% to 100%. Temperature and humidity can be set to any desired diurnal pattern giving considerable flexibility in experimental design. They have a roll-in-roll out capability which allows for multi-species use. Cattle can be housed in large single animal pens, metabolism crates or as a group (approx. 8 per room) depending on the nature of the study. The rooms are also set up to measure methane. Additional capacity to measure ammonia, CO_2 and O_2 are being installed in 2013.

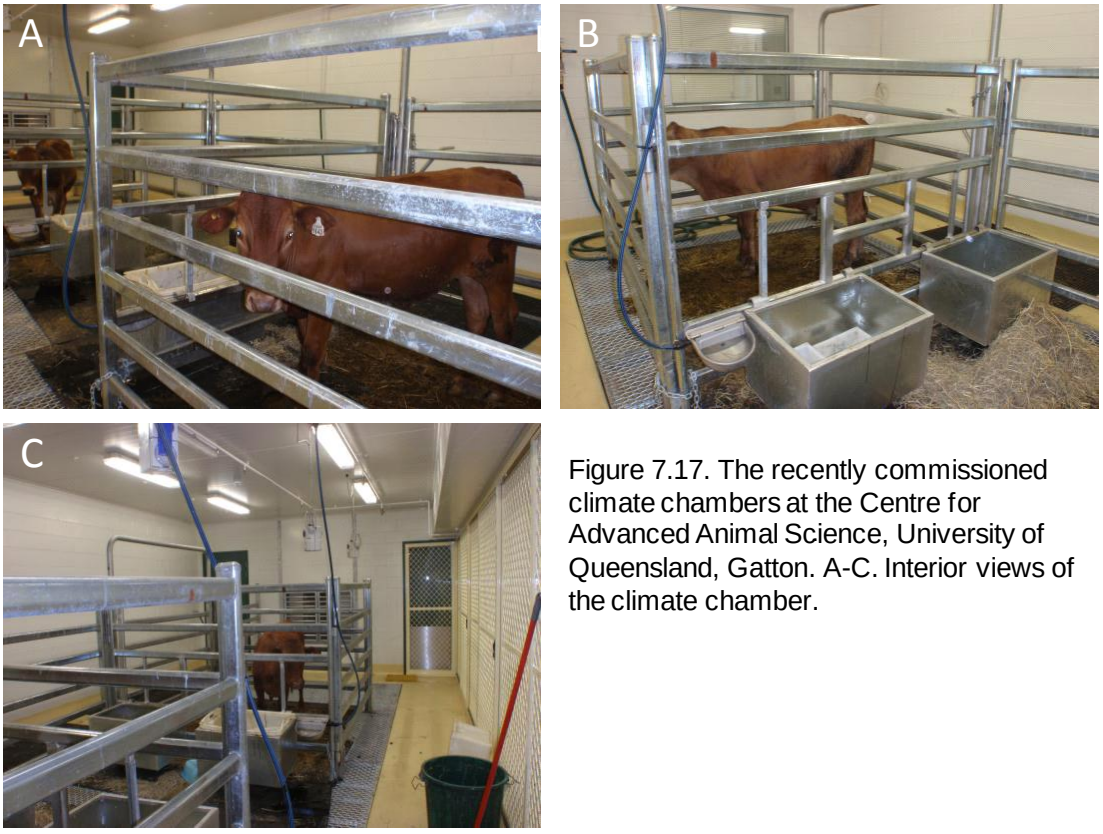

Figure 7.17. The recently commissioned climate chambers at the Centre for Advanced Animal Science, University of Queensland, Gatton. A-C. Interior views of the climate chamber.

In addition to the climate rooms, CAAS has 36 individual animal pens for controlled feeding studies and a 12 pen accredited research feedlot with weather station and capacity to remotely measure and record body temperature from cattle. The body temperature equipment is portable and can be used at any desired site.

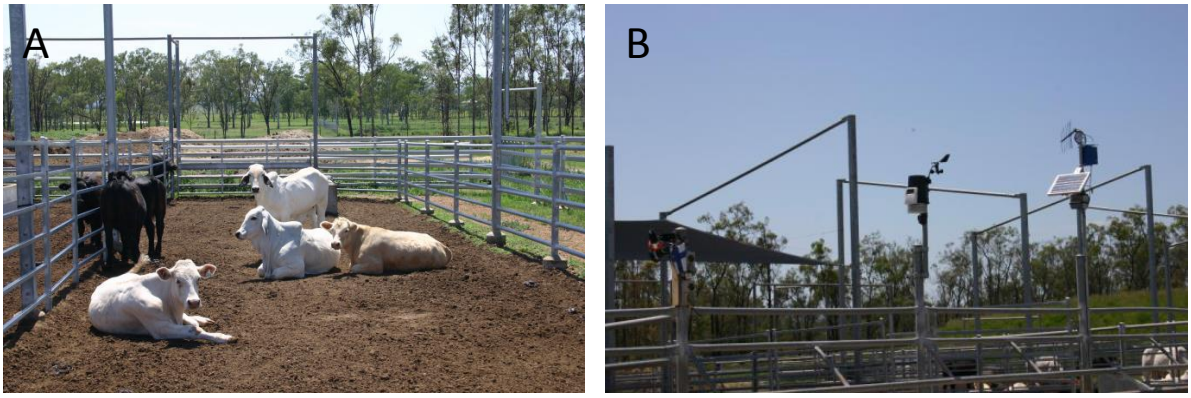

Figure 7.18. The new research feedlot at the Centre for Advanced Animal Science, The University of Queensland, Gatton. A. Cattle pens. B. Weather stations mounted over the research feedlot.

There are currently several MLA funded projects underway at CAAS. The feedlot is being used as part of a heat load study investigating the effects of night-time cooling on feedlot cattle and the impact of early morning weather conditions on heat balance in cattle. The climate rooms have had little use, but there are a number of projects that may be funded in 2013. The cost of using the climate rooms are currently $40 per room per day; the cost of using the feedlot is $15 per pen per day.

8.7.2 Department of Agriculture and Food Systems, University of Melbourne,

The University of Melbourne has two animal facilities that can accommodate climate experiments; the Parkville Animal Facility and at the Dookie College (located between Shepparton and Benalla, Victoria). Both facilities are designed for sheep experiments only.

The facility at the Parkville campus contains a single climate room which can be sub-divided into thermoneutral and heated sections by clear cafe style blinds. Thus, thermoneutral control animals are housed in the same room as those exposed to heat. The heated room consists of three walls of cafe blinds set against a concrete wall, and can be warmed to 40ºC. Blinds can be lifted at any time for the removal of animals and cleaning purposes. Humidifiers and heaters are used to maintain temperature within required limits. Five bar and two wall mounted heaters are used to increase ambient temperature within the heat room. Four humidifiers are used to help increase relative humidity within the heat room. Heat and humidity is monitored using temperature and humidity Dataloggers® placed in both the heat and thermoneutral sections. The heated section can accommodate six sheep in metabolism cages.

The Dookie College animal house facility also has a controlled temperature room designed to maintain temperature between 18ºC and 40ºC, using heaters, ceiling vents, and an air conditioner mounted on the rear wall. It is not humidity controlled. The climate room is able to house 6 sheep in metabolism cages.

8.7.3 Other Australian facilities

Over the years, various heat load experiments have been conducted in ruminants in other Australian facilities. The University of New England (Armidale, NSW) has climate rooms which accommodate sheep only. The now defunct Rendell Laboratories, the ex-CSIRO facility at Rockhampton, contained

a state-of-the-art cattle climate chambers in its time (1970-1980's) but these have been decommissioned. However, there is a move by CQU to revamp the facility. Murdoch University (Perth, Western Australia) possess a facility that suitable for cattle. No experiments have been conducted in this retro-fitted climate chamber since the early 2000's and currently is not serviceable for any new cattle work.

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10 Appendices

10.1 Appendix 1 Details on Measurement Devices

A brief outline of some of the technology (across species) that has been used is presented below.

Inter-reticulo/rumen telemetry bolus system

Gasteiner et al. (2009, *Wiener Tierärztliche Monatsschrift* 96, 188) used a wireless AD/C system that measured rumen pH as well as temperature. A similar device is available from Kahne Animal Health in NZ. (see Pinchak et al., 2010).

Rose-Dye et al. (2011, *Journal of Animal Science* 89, 1193) used a bolus from SmartStock LLC (Pawnee, OK). The bolus can be set to transmit rumen temperature at specified time intervals. A base station must be set up (about 2 hour work). It can transmit over 300 m from animal to base station, with repeaters give longer distance. Temperature measurements were confounded by drinking (although this could be useful if it allows drinking events to be determined).

Rectal temperature measurement – Gaughan et al. (1999) used rectal probes with a thermistor mounted into the tip and attached to a data logger (Smart Reader; ARC Systems, Brisbane). The probes were attached to the animal and the animal was restrained in a pen. The probes remained in place for up to 14 days. Reuter et al. (2010, *Journal of Animal Science)* used a home-made radiotelementry device needs to be tethered to resistant to expulsion during defecation (cost-$300).

Tympanic temperature measurement – Mader et al. (2010b) and Gaughan and Mader (2009) measured tympanic temperature of feedlot cattle in outdoor pens. Tympanic temperature was measured using Stowaway XTI data loggers and thermistors (Onset Corporation, Pocasset, MA, USA). The thermistors are placed on the tympanic membrane. The loggers remained on the animals for 6 days. These are inexpensive devices costing approximately $40 each. Tympanic temperature is very responsive to changing ambient temperature and has been used in a number of studies.

Vaginal temperature measurement – Numerous studies have been undertaken where vaginal temperature has been measured (Kendall et al., 2006; Tucker et al., 2008; Schütz et al., 2009; Vickers et al., 2010; Burdick et al., 2012). Vickers et al. (2010) compared rectal temperature to vaginal temperature in dairy cows. They reported a relationship between rectal and vaginal temperatures for fresh cows (n = 1,393; r = 0.81) and for peak-lactation cows (n = 556; r = 0.46). Burdick et al. (2012) reported a much better correlation between rectal temperature and vaginal temperature of r = 0.98.

Peritoneal temperature measurement - SubCue (Calgary, AB , Canada) mini data loggers were implanted into retroperitoneal fat body (visceral) (Henry et al., 2008, *Endocrinology* 149: 2019). Gaughan et al. (2010a) surgically implanted temperature transmitters (Sirtrack ltd, Havelock North NZ) into Angus between the internal abdominal muscle layer and the peritoneum at the right flank. The body temperature was highly correlated (r = 0.92) with rectal temperature.

Auditory Canal probes –infrared technology (e.g., Thermoscan ExacTemp IRT 4520; Braun, Boston, MA; AUR) and tympanic membrane temperature is considered to represent the temperature of the

blood to the pre-optic supraoptic region of hypothalamus responsible for controlling body temperature.

Non-invasive thermometry:

Rapid Accurate Temperature Establishment (R.A.T.E. ™ – US patent Medism USA Inc.) (http://www.medisim.co.il) is a handheld digital thermometer. Fundamentally, it measures the temperature on the surface of the skin, but also the heat flow. R.A.T.E™ monitors blood flow in the skin and calculates the temperature in the blood vessel.

Wearable remote monitoring devices – measure surface temperature only and are influenced and thus limited by environmental factors, hence unreliable.

Infrared emission – measures radiant heat from cutaneous vascular activity. This non-contact thermography provides sophisticated and complex imagery thus expensive, and confounded by environmental factors unreliable outdoors.

Vaginal Temperature Loggers

Brand	Model	Website	Logger Price	Reader & Software	Temp Range (°C)	Accuracy (± °C)	Resolution (°C)	Length (cm)	Diameter (cm)	Weight (g)	Battery (years)
Vemco	Minilog-TX	vemco.com	Discont.	Discont.	-30 to 40	0.2	0.1	7	1.6	23	5
Vemco	Minilog-8	vemco.com	Discont.	Discont.	0 to 42	0.3	0.2	9.2	2.0	40.5	-
Vemco	Minilog-II-T	vemco.com	$286	$780	-30 to 80	0.1	0.01	9.8	2.3	52.2	10
Star Oddi	DST micro-T	star-oddi.com	$324	$600	5 to 45	0.2	0.03	2.54	0.83	3.3	1.5
HOBO	TidbiT v2 (UTBI-001)	onsetcomp.com	$133	$109	-20 to 70	0.2	0.02	1.7	3.0 x 4.1	19.6	5
HOBO	Water Temp Pro v2 (U22-001)	onsetcomp.com	$123	$109	-40 to 70	0.2	0.02	11.4	3.0	42	6
HOBO	U12 / U12-015	onsetcomp.com	$259	$109	-40 to 125	0.25	0.03	10.2	1.75	72	3
Thermochron	iButton - DS1922L-F5#	embeddeddatasystems.com	$46	$55	-40 to 85	0.5	0.06	0.5	1.6	3.3	7
Thermochron	iButton - DS1921H-F5#	embeddeddatasystems.com	$22	$55	-15 to 46	1.0	0.125	0.5	1.6	3.3	10

Table 8.1 Characteristics of temperature loggers used by Dr Cassandra Tucker and her team at UCDavis.

10.2 Appendix 2 Preparatory Questions Forwarded to Overseas Colleagues

Meat and Livestock Australia have “undertake a well-researched, systematic and coordinated assessment of nutritional strategies that can be employed to assist with management of heat load in feedlot cattle”. Thus, the purpose of our visit is to gather information that can contribute to this comprehensive review of heat stress nutrition. We are also tasked with proposing a 5 year R&D program on heat stress nutrition, and knowledge of international expertise, direction of research and opportunities to collaborate will be key to this plan.

Specifically, we are interested in

- Your most current research (that you are willing to share with us) and where this work has taken your thinking;
- Knowledge gaps that you have identified in heat stress nutrition
- Knowledge that has reached consensus
- What experiment(s) would you do if you could
- Use of metabolic and endocrine and cellular measures of heat stress
- Your understanding of advances by the feed manufacturers
- The future of heat stress research in your region/country
- What skills sets or disciplines are under-utilised or under threat that could impact on future heat stress research and its nutrition

We are also keen to understand your experimental set up

- Animal experimentation skills and experience (especially in cattle)
- Temperature, humidity and airflow control of climate controlled rooms, and recording of the microclimate. Experience in maintenance and repair of equipment. Consistency of microclimate between rooms
- Research feedlots and their instrumentation
- Capacity of facility (how many animals? What size/weight of animal? Ease of access to animals, comfort of animals)
- Record experience of use of transmitting core body temperature devices.
- Ability to included room atmosphere CO_2(g) measurement.
- Veterinary support
- Animal ethics protocols and procedures