Chapter 1 General Introduction

Climatic factors, such as solar radiation, wind, rainfall and humidity, influence the environment where an animal lives and affect its behaviour and physiology. To survive, animals must adjust to environmental changes through acclimatisation. Failure to acclimatise results in consequences such as lower productivity, greater susceptibility to diseases, and can be fatal. Failure of animals to adjust to the climate can therefore lead to financial losses.

Norris (2005) reported that the numbers of sheep exported from Australia to the Middle East between 1985 and 2004 was above 3 million per annum (2005) and mortality ranged from 0.71% (Fremantle in 2004) to 2.1% (Portland in 2002). Failure to eat and salmonellosis were the two main causes that together were responsible for 75% of total deaths. High ambient temperature and relative humidity were two of the predisposing factors identified as causing the mortalities. Climatic conditions affecting the live exports and mortality data have been reported by Black *et al.* (1994) and Norris and Richards (1989a). The maximum and minimum ambient temperatures and relative humidity on the voyages was 20 and 30°C and 70 and 90%, respectively (Black *et al.* 1994). The average ambient temperature during the summer in the destination areas of live exports, especially in the Middle East, was up to 50°C during summer and above 30°C during winter with relative humidity above 70% (Humphreys *et al.* 2003). 70% humidity must be specific to gulf country.

Three proposed management strategies that can minimise the effect of thermal stress are: physical modification of the environment, genetic development of heat-tolerant breeds, and improved nutritional management practices (Beede and Collier 1986). In this study, nutritional strategies, particularly the use of feed additives and manipulation of drinking water temperature were investigated. Various feed additives that have been considered as a means of alleviating heat stress in livestock including oligosaccharides, probiotics and betaine (Fernandez *et al.* 2004; Kumagai *et al.* 2004; Newman *et al.* 1993). One commercially produced additive is a yeast-based feed additive, 'Farmer-Peck' (in this thesis referred to as 'yeast-based feed additive'). Manipulation of water temperature is also a potential nutritional management strategy for heat-stressed sheep (Pond *et al.* 2005).

Two experiments were conducted at the School of Rural Science and Agriculture, with the approval of the Animal Care and Ethics Committee, the University of New England in Australia. The objectives were to evaluate the effects of the yeast-based feed additive and different drinking water temperatures in Merino sheep in cool and hot climatic conditions.

In the first experiment, Merino sheep were offered lucerne pellets without and with the yeast-based feed additive. In the second experiment, Merino sheep were offered lucerne chaff and drinking water at different temperatures. Both experiments were conducted in climate rooms maintained to create heat-stress conditions (up to 40°C) and comfortable conditions (20°C). Body temperature, behavioural, metabolic and physiological responses, and production measures were recorded. Animal responses recorded in the first experiment were body temperature, respiratory rate, feed and water intake and feed digestibility and in the second experiment, feed and water intake, water preference, digestibility of the feed and nitrogen balance were recorded.

The literature review in Chapter 2 examines the current state of knowledge on the effects of heat stress in ruminants. Experimental materials and methods, especially laboratory analyses are discussed in Chapter 3. Chapters 4 and 5 are the reports of the two experiments detailed above, with their respective results and discussions. A general discussion of the experiments and the implications of the findings are presented in Chapter 6.

Chapter 2 Literature Review

The purpose of this review is two-fold. First, the intention is to establish an improved understanding of animal (especially sheep) heat-loss mechanisms under hot climatic conditions, the current understanding of respiration rate, body temperature (skin, rectal and tympanic temperature), feeding behaviour, water intake, digestive responses, and excreta production of sheep subject to hot climatic conditions. Second, there is a discussion of heat-stress management strategies that can be used to help animals to cope with high ambient temperature conditions.

2.1 Characterisation of animal responses to environmental conditions

2.1.1 Acclimation, acclimatisation and adaptation

The capability of animals to adapt physiologically and biochemically to new environmental conditions is known as acclimation (Horowitz 2003). Acclimation is usually describes a physiological change occurring in experimental treatments, especially under controlled climatic conditions (Johnson 1987a; Schmidt-Nielsen 1991). Lagerspetz (2006) provided an additional view about thermal acclimation. Compiled from the work of other authors, he defines acclimation as an adaptive change, a compensatory response due to a change of environmental variables during the lifetime of an organism (Lagerspetz 2006).

Unlike acclimation, acclimatisation only occurs naturally (Schmidt-Nielsen 1991). Acclimatisation to heat can be described as a number of physiological adjustments which occur, for example, when an animal that is used to living in a cool climate is suddenly moved to a hot climate (Leithead and Lind 1964). Acclimatisation, caused by stressful changes in seasonal or geographical factors, reduces stress on the animal (Johnson 1987a) by slowly bringing about physiological and biochemical changes, which affect breathing, circulation and blood chemistry (Kumar *et al.* 2003; Stewart 1991).

Adaptation describes an animal's response to a stressful component of the total environment, resulting in a change that reduces the physiological stress experienced by the animal (Johnson 1987a). To survive and be productive an animal must reduce

environmental stress by modifying its physiological, anatomical and behavioural characteristics (Ledezma 1987).

Physiological and anatomical characteristics that are associated with adaptation have been studied. The cardiovascular system and the sweating mechanism are two physiological adaptations in animals (Johnson 1971; Leithead *et al.* 1964). The physiological adaptation for temperature control, as a response to a change of environment, has been explained by several authors (Currie 1988; Stewart 1991). Growth of very thick wool by sheep in cold and damp climates is an example of anatomical adaptation in the animal assisting acclimatisation to a cool environment (Horowitz 2003).

Animals also show behavioural adaptations to changes in the environment. Sheep seek shade during the day (Johnson 1987b) and eat less and drink more during the hotter hours (Hogan *et al.* 2004). Some behaviours in sheep, such as vocalising, panting, and moving more frequently, are indicators of heat stress (Cockram 2004). Nienaber and Hahn (2000) noted that responses of livestock to hot, humid conditions include seeking shade, staying around water sources, increasing respiration rate and reducing DM intake (DMI).

2.1.2 Thermoregulation

Acclimation, acclimatisation and adaptation are all homeostatic mechanisms (Currie 1988) enabling the animal to maintain its body temperature in the thermo-neutral zone, i.e. the narrow range of ambient temperature where core body temperature can be held constant (Currie 1988; Ingram and Mount 1975) (Figure 2.1). The ability of an animal to maintain its body temperature within a thermo-neutral (CD) zone is known as thermoregulation (Currie 1988). Hyperthermia (F zone) occurs when the animals' body is unable to maintain a normal temperature and its temperature increases significantly above normal. The opposite condition is hypothermia (A zone) (Currie 1988; Ingram *et al.* 1975).

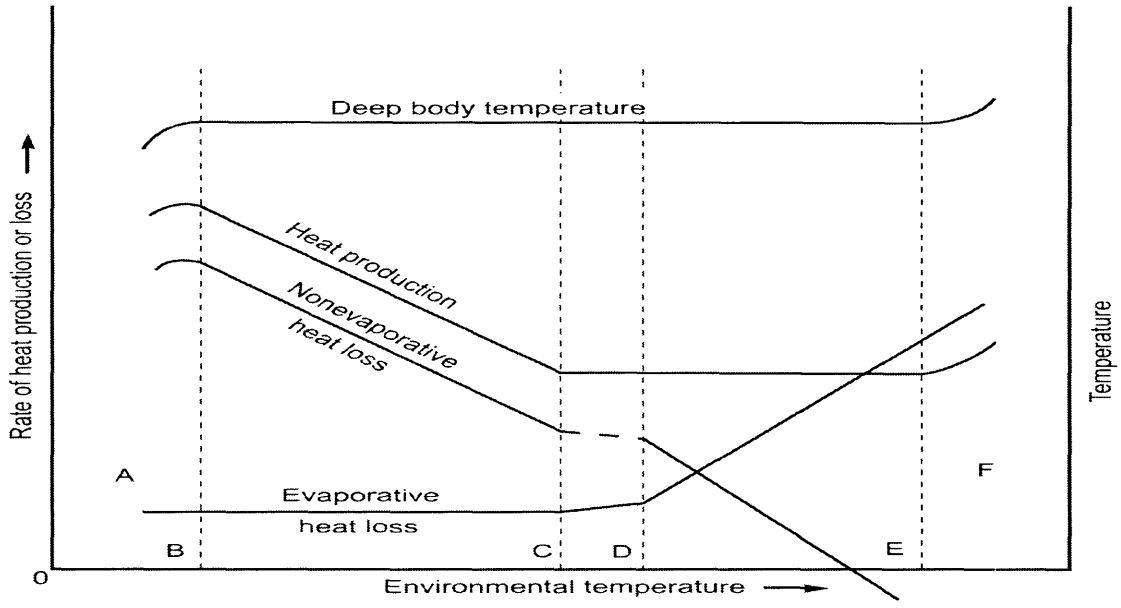

Figure 2.1 Diagram showing the relationships between the deep body temperature, total, sensible, and latent heat loss in a homeothermic animal due to the environmental temperature (after Ingram *et al.* 1975).

A: Zone of hypothermia; B: temperature of maximum metabolism and beginning hypothermia; C: lower critical temperature; D: temperature of marked increase in latent heat loss; E: temperature of beginning hyperthermia; F: zone of hyperthermia, CD: thermo-neutral zone or comfort zone; CE: zone of minimal metabolism; BE: thermoregulatory range; 0: zero point; Y: rate of heat production or loss; Z: temperature

In a homoeothermic animal, a dynamic state exists between internal and environmental temperature, known as homeostasis (Ingram *et al.* 1975). The ruminant maintains a relatively constant core body temperature around 38°C (NRC 1981). There is continuous heat production in the animals' body in order to keep this body temperature constant. Heat is released when the feed nutrients are metabolised in body tissues (McDonald *et al.* 2002) and the metabolism of gut microorganisms also generates heat. Heat produced from this source is as much as 7 - 8 % of the metabolisable energy (ME) intake (McDonald *et al.* 2002). Chemical energy (in the form of solid, liquid and gas) and heat (known as the heat increment of food) are produced after feeding. Heat increment can be observed in fasting animals that show an increased abdominal temperature within a few hours of feed ingestion (McDonald *et al.* 2002). McDonald *et al.* (2002) explained that the heat increment was a consequence of ingestion of feed and the metabolism of absorbed nutrients. Another source of energy is muscle contraction that can be observed as the animal is shivering (Reece 2005) or when the animal is

active (Entin *et al.* 1999). Ambient air and surroundings can also be a source of body heat as shown by studies in sheep (Macfarlane *et al.* 1960; Sevi *et al.* 2001).

Heat transfer between the animal body and the environment

The concepts of acclimation, acclimatisation and adaptation are based on the heat gain, heat balance and heat loss principles (Yousef *et al.* 1968). Heat is transferred between an animal and its surroundings through convection, conduction and radiation, and the evaporation of water from the skin and respiratory passageways (Ingram *et al.* 1975; Monteith 1973; Reece 2005). The types of heat transfer are functions of the characteristics of both the animal and the environment (Table 2.1).

Table 2.1 Factors influencing the different types of heat transfer between organism and environment

Type of transfer	Animal characteristics	Environment characteristics
Radiant	Mean radiant temperature of surface; effective radiating area; reflectivity and emissivity	Mean radiant temperature; solar radiation and reflectivity of surroundings
Convective	Surface temperature; effective convective area; radius of curvature and surface type	Air temperature; air velocity and direction
Conductive	Surface temperature; effective contact area	Floor temperature; thermal conductivity and thermal capacity of solid material
Evaporative	Surface temperature; percentage wetted area; site of evaporation relative to skin surface	Humidity; air velocity and direction

Radiation is an important means of heat transfer as shown by a study on cattle carried out in an equatorial location at noon. Briefly, from total heat gain of 688.7 W/m^3 (50.7 W/m^3 from metabolic heat and 638 W/m^3 from radiant heat), 57.7% of the heat was radiated (Robertshaw and Finch 1984). Sheep carrying 4 -7 cm wool and exposed to sun (38.8°C) and wind were estimated to receive 6281 kJ/day of heat by radiation (Macfarlane *et al.* 1960).

Convective losses involve heat transfer to a flowing medium (usually air for animals) providing a continuous heat gradient from the animal's skin to the environment (Currie

1988). Conduction occurs when regions with greater molecular kinetic energy pass that energy to regions with less molecular energy (Johnson 1987a). Higher water content in muscle and skin raises the conductivity of these organs to 30 times that of air (Currie 1988). Evaporation through the skin and lungs of animals in a hot environment also releases heat (Johnson 1987a).

The body strives to keep a constant core temperature. This may be achieved at the expense of the skin temperature (Ruckebusch *et al.* 1991), which may be permitted to vary in order to assist the core temperature to remain constant. So, for example, as body temperature rises toward hyperthermia, the animal automatically reacts to return it to normal (Stewart 1991).

Climatic factors

Meteorological factors, such as radiant energy, ambient temperature, wind, and relative humidity, contribute to the heat status of animals (West 1999). Radiant energy from the sun affects body temperature. Sevi *et al.* (2001) exposed lactating ewes to the sun in a Mediterranean region where the mean intensity of solar radiation ranged from 229 to 331 W/m^2 and the maximum ambient temperature was 3.9 to 6.8°C higher than that in the shade. This heat load was enough to increase rectal temperature by 0.4°C and respiration rate by 27.5 breaths per minute (bpm) among the ewes.

An animal suddenly subjected to excessive ambient temperatures experiences an increase in body temperature (Currie 1988). Hogan *et al.* (2004) reported a linear increase in sheep body temperature from 39.3°C to 39.9°C when ambient temperature increased from 20°C to 35°C.

A hot plate apparatus developed by Bennet and Hutchinson (1964) revealed how one climatic factor, the wind, affects the insulation of fleece and heat transfer. The heat transfer coefficient of sheep ($kcal/m^2.hr.°C$) increased linearly at any length of fleece (none, 0.48 cm or 1.27 cm) as the air movement increased from 7 to 200, 7-300 or 7-470 cm/sec. Bhatta *et al.* (2005) reported that, during the monsoon season, sheep exhibited greater heat stress than in summer even though the ambient temperature during the monsoon season was lower. The higher relative humidity in monsoon was the climatic factor responsible for the difference.

Thus, two main climatic factors, ambient temperature and relative humidity, need to be assessed when measuring the effect of climatic conditions on animal heat stress. They

can be calculated as a temperature humidity index (THI), and THI has been used for determining the heat-stress levels in dairy cows (Armstrong 1994) and sheep (Silanikove 2000). The THI can be calculated from a combination of wet and dry bulb air temperatures and expressed as:

$$THI = 0.72 (W^{o}C + D^{o}C) + 40.6$$

where $W^{o}C$ = wet bulb and $D^{o}C$ = dry bulb.

THI values of 70 or less are considered comfortable, 75-78 stressful, and values greater than 78 cause extreme distress and animals are unable to maintain thermoregulatory mechanisms or normal body temperature (Silanikove 2000).

Microclimate modification for animals in hot climates

Microclimate manipulation could be applied either directly to the body of animal, as in shearing (Pennisi *et al.* 2004) and wetting (Gaughan *et al.* 2004; Musimra *et al.* 1987), or indirectly to the controlled environment by the use of fans for cooling in the hot season or heaters in the cool season (Qvarnstrom 2002). Environmental manipulations, such as providing shade, maximising air movement, providing cool drinking water, designing sprays and sprinklers to reduce absorption of radiation, and maximising heat loss via conduction, convection and radiation, are used to modify natural behaviour (Beede *et al.* 1986; Gebremedhin and Wu 2002; Johnson 1987b; Lofgreen *et al.* 1975).

Cooling can be provided by wetting cattle, which provides cooling as the water evaporates, or through evaporative cooling of the air or the surrounding surfaces (Gebremedhin *et al.* 2002; Hahn 1985). A study of wetting in the Merino sheep was conducted by James *et al.* (1984). These workers reported the thickness of the skin wax layer was decreased due to wetting but this technique probably has little application to woolly sheep.

2.2 Respiration rate

Respiration is a means of exchanging gases (oxygen and carbon dioxide) and also a means of heat exchange between an animal and its environment (Frandson 1981). The respiratory passages and skin can work effectively to eliminate the heat produced by metabolic processes only if the ambient temperature is similar to the skin temperature of the animal (Blaxter 1962). When the ambient temperature rises above that of the skin, respiration rate increases.

The normal respiration rate of sheep ranges from 10 to 40 bpm, but the maximum respiration rate can reach 350 bpm (Adams and McKinley 1995). These values change with ambient temperature (Hogan *et al.* 2004; Maloiy and Taylor 1971), physical activity, fleece cover (Macfarlane *et al.* 1966) and water supply (Lynch *et al.* 1972). Respiration rate of Merino sheep was 100, 132, 184 and 216 bpm, respectively when they were kept in ambient temperatures of 20°C, 25°C, 30°C and 35°C (Hogan *et al.* 2004). Maloiy *et al.* (1971) found a respiration rate of East African goats of 20 bpm in an ambient temperature of 20°C and respiration rate increased to 250 bpm as the ambient temperature was increased above 40°C. A lower length of wool affects respiration rate. Macfarlane *et al.* (1966) reported that under the sun at ambient temperature of 36 or 37°C, respiration rate of woolly sheep was 137 bpm. After shearing, the respiration rate of shorn sheep was 178 bpm higher than in unshorn group.

A finding reported in conjunction with water intake was that, at 14°C, the group of sheep on a restricted water regime had a respiration rate of 47 bpm which was lower than that in a group with unlimited water (129 bpm). This pattern remained the same as the ambient temperature was increased to 32°C, when the restricted water group had a respiration rate that was 133 bpm higher than that of the unlimited-water group (182 bpm) (Brown and Lynch 1972).

The surfaces of the upper respiratory tract are cooled by convection and evaporation during inhalation and exhalation (da Silva *et al.* 2002). As inhaled air flows through the passages, it gains heat and water vapour and becomes saturated. On exhalation, heat and water are released so as to approach the ambient temperature (Stewart 1991). If the ambient temperature is higher than that of the exhaled air, the increased uptake of oxygen may build up the heat load of sheep (Taniguchi *et al.* 2004). These conditions will counteract the main function of evaporative cooling through the surfaces of the respiratory passages (Yousef *et al.* 1968).

Under hot conditions, heat loss from the animal depends on both respiratory and cutaneous evaporation (Johnson 1991). The percentage of heat dissipated through respiratory and cutaneous evaporation varies, depending on breed and environmental conditions (Table 2.2).

Small ruminants, compared to cattle, rely more heavily on respiratory mechanisms for cooling when exposed to hot conditions (Table 2.2). Some species depend primarily on

panting for increased heat loss whereas others rely on a small increase in respiratory depth and frequency (Bianca 1965; Johnson 1987a).

Table 2.2 The percentage of heat dissipated through respiratory and cutaneous evaporation by various ruminant species under hot conditions

Breed and reference	Percentage of evaporation	
	Respiratory	Cutaneous
Boran steer (*Bos indicus*) (Finch 1986)	16	84
Bedouin goats (McGregor 2004)	33	67
Australian Merino sheep (Blaxter 1962)	25 – 35	65 – 75

2.3 Skin temperature

2.3.1 Skin temperature as an indicator of heat stress

Skin temperature is one of the physiological measurements used to assess the degree of discomfort of a heat-stressed animal (Stewart 1991). Skin temperature of ewes in thermo-neutral conditions (20°C) was significantly ($P < 0.01$) lower than that of ewes in conditions sufficient to cause heat stress (Abdalla *et al.* 1993). The higher skin temperature of the ewes in the hot conditions occurs because the blood is carrying heat from within the body to dissipate it on the surface (Leithead *et al.* 1964).Also, the skin may absorbs heat from direct solar radiation.

Care should be taken, however, when skin temperature is used as an indicator of heat stress because sometimes the data can be unrealistic. For example, skin temperature of Merino sheep was reported to reach a maximum of 55°C during summer (Sherwin and Johnson 1990). However, Johnson (1991) reported a lower mean skin temperature difference between sheep exposed to the sun and sheep resting in the shade (44.8°C vs. 43.4°C). An explanation raised by Sherwin and Johnson (1990) to explain the unrealistically high skin temperature of 55°C was that the temperature sensor used to record skin temperature occasionally received direct radiation from the sun as the animals altered their posture.

2.3.2 Anatomy of the skin and the importance of pelage

The role of skin and fleece in heat transfer in sheep has been studied extensively (Bennett *et al.* 1964; Pennisi *et al.* 2004; Piccione *et al.* 2002a). The skin layers function to cover the body and provide protection, awareness of the environment, excretion and thermoregulation (Hafez 1968c). The degree of melanin pigmentation of animal skin can be associated directly with the climate, and is especially affected by solar radiation at high altitude. Animals inhabiting warm and humid regions show greater pigmentation than those found in cooler and drier areas (Hafez 1968b).

The skin has several layers, epidermis, dermis and hypodermis (subcutaneous) which function differently with regard to the ambient temperature (Reece 2005). Hafez (1968c) illustrated the skin in five layers: skin surface, epidermis, melanin, dermis and subcutaneous fat. In his diagram, Hafez (1968c) has shown that nerve fibre receptors and skin glands were located in the epidermis, while blood vessels were found in the dermis.

2.3.3 Heat exchange by evaporation from the skin

Evaporative heat loss from the skin is higher than that from the lungs in heat-adapted animals (Table 2.2). The rate of heat loss is determined by the difference of the vapour pressure between saturation at skin temperature and the ambient temperature. If the skin temperature is rising because ambient temperature has increased, then water losses through the skin are increased (Mount 1968). Increasing the relative humidity of the air reduces the water vapour gradient between the skin and the air, and thereby depresses evaporation (Bianca 1968). Air movement is important to allow efficient removal of water vapour on the skin (Ingram *et al.* 1975) and increased air velocity increases heat dissipation from the wet skin (Gebremedhin *et al.* 2002).

Sweating is a physiological thermoregulatory response of animals to high ambient temperatures (Silanikove 2000). Many animals secrete aqueous solutions onto the skin surface where evaporation can take place (Currie 1988). Johnson (1971) reported the amount of sweat discharged by unshorn Welsh mountain sheep at ambient temperatures of 20, 30 and 40°C was 0.03, 0.48 and 5.06 $g/m^2.h$, respectively. For their shorn counterparts at the same ambient temperature, the values were 0.29, 5.84 and 9.27 $g/m^2.h$. This study showed how the body loses heat through sweat at high ambient

temperature. Shearing helps the sheep to excrete more sweat and therefore more heat is dissipated.

In cool climates, there is unavoidable heat loss by evaporation that occurs through the lungs and because of diffusion of water through the skin. This evaporation process is known as 'insensible perspiration' (Reece 2005). As the climate becomes warmer, the amount of water lost by insensible perspiration increases slightly until active sweating starts (Leithead *et al.* 1964). Water can be excreted through skin that has no sweat glands because the skin is not completely impermeable to water. Moreover, because the rate of water loss through the skin depends partly on the surface temperature of the epidermis, moisture vaporisation increases when an animal vasodilates in response to heat (Ingram *et al.* 1975).

2.3.4 The influence of blood on heat loss at the skin surface

Blood has a major role in moving heat around the body. Because circulating blood is a distributor of body heat, heat can be lost from blood if it is brought close to a cooler skin surface (Reece 2005). The cardio-vascular system delivers heat to the skin from where it is dissipated (Horowitz 2003). Heat loss at the skin is increased when blood passes through arterio-venous anastomoses that are close to the body surface (Stewart 1991).

Haematocrit, usually known as packed cell volume (PCV), and heart rate can both be used as indicators of heat-stress in sheep. Because evaporation brings about water loses, as the blood passes the body surface and evaporation occurs, the haematocrit is increased. Heart rate increases in hot conditions to pump the blood into the skin surface where evaporation takes place. Haematocrit and heart rate values for Comisana lambs (Pennisi *et al.* 2004) indicate the significance of blood flow in animals experiencing heat stress.

2.4 The influence of wool on thermoregulation

The insulation of animals is affected by three elements: a layer of tissue, fat and skin; a layer of relatively still air that is trapped within the fleece and an outer boundary layer (Monteith 1973). The basic function of fleece is to provide an insulating layer to protect the animal against hot and cold conditions. It emits long-wave radiation and insulates

against incoming heat by conduction (Macfarlane *et al.* 1966; Pennisi *et al.* 2004; Priestley 1956).

Wool presents a barrier between the environment and the animal, so if the ambient temperature increases, the wool temperature increases too (Costa *et al.* 1992). One study reported that rectal temperatures for shorn sheep are lower (38.78 vs. 39.79°C) than those of unshorn sheep (39.33 vs. 39.96°C) in ambient temperatures of 10.5 – 25°C and 25 – 46.5°C, respectively (Silva *et al.* 1992). Piccione *et al.* (2002b) recommended shearing to reduce heat stress in Comisana ewe lambs.

The growth of wool is affected by nutrition and ambient temperature. Dixon *et al.* (1999) found that fish meal caused wool to grow faster than did barley grain. Thwaites (1967) concluded wool growth rates were depressed by heat stress at 40.5°C compared with control sheep housed at 6.7 to 17.2°C and this was probably mainly due to reduced feed intake.

The surface temperatures of the fleece of shorn and unshorn sheep differ. One study tested the effect on the skin temperature of a sheep after removing its fleece. Measuring the sheep's wool temperature while it stood in the afternoon sun revealed that the gradient across the remaining 5 – 8 mm of wool was reduced to 4°C. This study suggests that the dark matted wool tip functions to absorb or to radiate solar energy (Macfarlane *et al.* 1966). These workers measured the surface and skin temperature on the backs of sheep standing in the sun at a dry bulb temperature of 37°C. These workers found that the skin temperature of shorn sheep was 2°C above that of unshorn sheep. Another study, conducted to determine the effect of shearing on the thermal homeostasis of female Comisana lambs during summer, indicated that rectal temperature did not differ between the shorn (39.5±0.4°C) and the unshorn group (39.8±0.3°C) 80 d after shearing (Pennisi *et al.* 2004). However, the respiration rate at the same time was higher in the unshorn group (105 vs. 68 bpm) and heart rate was also higher (119 vs. 102 bpm). In contrast, the haematocrit value was found to be higher in the shorn group (38 vs. 32%), so that the percentage of oxygen saturation in the blood (SpO_2) of the shorn group was higher (97.4 vs. 89.3%) (Pennisi *et al.* 2004). Pennisi *et al.* (2004) concluded that shearing ewe lambs resulted in a lower heat stress during the summer even though the shearing did not apparently influence thermal homeostasis.

The increase in skin temperature of shorn sheep seems to affect the animals' behaviour. For example, Macfarlane *et al.* (1966) showed that sheep seek shade after shearing. Johnson (1991) concluded that the effect of shade depended, in part on, how much fleece the sheep were carrying. These studies show that wool plays an important role in the thermoregulatory system of a heat-stressed animal.

2.5 Rectal and tympanic temperatures

Core body temperature measured from organs or tissues, such as the liver, brain, intra-ruminal site or carotid blood, varies and is usually higher than the rectal temperature (Anderson and Jonasson 1993; Blaxter 1962; Reece 2005). This difference is largely caused by metabolic rate (Blaxter 1962; Reece 2005), blood flow and distance of the tissue from the surface (Reece 2005) and extra heat produced by rumen micro-organisms (Anderson *et al.* 1993).

Nevertheless, while rectal temperature does not always accurately represent a mean of deep body temperature, the change in rectal temperature has frequently been used as an indicator of physiological adaptation to a tropical environment (Yousef *et al.* 1968). This is because rectal temperature reaches equilibrium more slowly than temperatures at many other internal sites, such as central vessels and it is a good index of a true steady state (Anderson *et al.* 1993; Reece 2005).

The normal range of rectal temperature is species-specific. In sheep, the normal range is 38.3 – 39.9 °C with an average of 39.1 °C (Anderson *et al.* 1993; Gregory and Grandin 1998). Any rectal temperature which exceeds the normal range indicates hyperthermia (Blackshaw and Blackshaw 1994; Gregory *et al.* 1998) or should be considered as a sign of severe heat stroke requiring prompt treatment (Blackshaw *et al.* 1994).

Many studies have established the ability of various domestic animals to withstand external heat (Anderson *et al.* 1993). In sheep, the rectal temperature starts to rise above normal at an air temperature of 32°C, and panting at the mouth begins when rectal temperature reaches 41°C. Unless the relative humidity is high (above 65%), the sheep can withstand an external temperature as high as 43°C for several hours (Anderson *et al.* 1993). In the post-discharge phase of the live export to the Middle East region, however, sheep may be subjected to more extreme climatic conditions such as those

reported by Anderson *et al.* (1993) which lasted for an average of 15 d (D.B. Savage pers. comm. 2006).

2.5.1 Factors that influence rectal temperature

Factors affecting rectal temperature have been studied, including circadian and seasonal variation (Silva and Minomo 1995), shearing and breeds (Piccione *et al.* 2002a), housing (Bhatta *et al.* 2005), physical activity (Entin *et al.* 1999) and feed consumption and drinking water temperature (Bailey *et al.* 1962).

Regardless of time of day or season, researchers agree that rectal temperature appears to be closely correlated to ambient temperature (Dixon *et al.* 1999; McCrabb *et al.* 1995; Neiva *et al.* 2004). Sheep in a hot environment (32 – 40°C, RH 50 – 70% and THI 83 – 88) have a higher rectal temperature (40.1°C) than those maintained in a cool environment (13 – 15°C and THI 56 – 58; rectal temperature 39.2°C) (Dixon *et al.* 1999).

Shinde *et al.* (2002) found the physiological state of goats, measured by rectal temperature, varied with season as well as diurnally. Santa Ines sheep in the northeast of Brazil for example had higher rectal temperatures in the afternoon (39.1°C) than in the morning (38.9°C) (Neiva *et al.* 2004). In contrast, the rectal temperature of sheep housed in sheds and in open corrals in a semi-arid region of India was similar during the morning (0600 h) and the evening (2100 h) throughout all seasons. The temperature humidity index, however, was significantly different in the monsoon, winter and summer seasons (Bhatta *et al.* 2005). In another study (McCrabb *et al.* 1995) in the semi-arid tropics of northern Australia, Merino sheep had a constant rectal temperature during summer for three successive years. McCrabb *et al.* (1995) concluded rectal temperature measurement was an effective tool to assess the level of hyperthermia and to predict the animal productivity in the following years.

Apart from the effect of ambient temperature, feeding the animal with different types of feed, especially concentrates, can also affect the rectal temperature (Neiva *et al.* 2004). When Santa Ines sheep were fed 70% concentrates, their rectal temperatures were higher (39.2°C) than those fed 30% concentrates (38.8°C) (Neiva *et al.* 2004). A study of desert goats by Ahmed and El Kheir (2004) showed that the use of feed, availability of food and water influenced rectal temperature (Table 2.3). Goats offered lucerne hay had higher rectal temperatures in the morning and in the afternoon than those offered

grass hay. Restricted water and feed did not affect rectal temperatures of the goats in the morning. In the afternoon, restricted water had no effect on rectal temperature of the goats offered either lucerne hay or grass hay. In contrast, when feed restriction applied, goats offered lucerne hay exhibited higher rectal temperatures than their counterparts offered grass hay. Dixon, *et al.* (1999) concluded that rectal temperature was not affected (P>0.05) by the provision of feed supplements. The different results recorded by Neiva *et al.* (2004) and Dixon *et al.* (1999) were possibly dependant on the type of feed used, and so concentrates and supplements that require different digestive and metabolic processes (Pond *et al.* 2005).

Table 2.3 Effects of water (40% of *ad libitum* intake) and food restriction (to 18% of *ad libitum* intake) on rectal temperature in desert goats fed lucerne hay and grass hay (Ahmed and El Kheir, 2004)

	Ad libitum water and food	Restricted water	Restricted feed
Rectal temperature (°C) at 0800 h			
Lucerne hay	38.85^{A}	38.66^{A}	38.3^{A}
Grass hay	37.89^{B}	38.64^{B}	37.56^{B}
Rectal temperature (°C) at 1300 h			
Lucerne hay	39.34^{A}	39.35^{a}	39.01^{a}
Grass hay	38.80^{B}	38.61^{a}	34.45^{b}

Values are means of 9 animals

Values within the same column bearing different superscripts differ significantly: ^{AB} P<0.01; ^{ab} P<0.05.

2.5.2 Tympanic temperature

In general, the capacity for increasing blood flow by vasodilation is highest in body parts having a high surface/volume ratio, such as the ears, the legs and the tongue. These parts usually have no hair or less hair cover than the body, so that heat is easily dissipated from them to the environment (Bianca 1968; Monteith 1973). Some animals

subjected to heat stress dissipate large amounts of heat through their ears (Monteith 1973). The vascular supply of the ear contains arteriovenous anastomoses that allow increased quantities of warm blood to be shunted rapidly through the ear (Currie 1988; Hafez 1968b). Mean ear surface temperatures of sheep were reported to be higher in both high and cool ambient temperature (Woodgate *et al.* 2001). These workers simulated summer-autumn rainfall and wind effects and found they decreased ear surface temperature of sheep (Woodgate *et al.* 2001). These results indicate that ear surface temperature is not likely to be a good indicator of core temperature.

In practice many researchers use tympanic temperature measurements instead of ear skin temperature measurements (Davis *et al.* 2003; Drew 1996). The temperature of the central nervous system, and the brain in particular, is the most closely regulated part of the body, as the function of the brain is very susceptible to temperature changes. Tympanic temperature has been used as an indication of core temperature because the tympanic membrane and the brain blood supply share the same origin (Robertshaw 1985). Tympanic temperature, measured by sensors secured in the ear canal, has been shown to be a relatively sensitive measure of thermoregulatory responses (Hahn 1999). It also serves as an index of core temperature and hypothalamic temperature (Brinnel and Cabanac 1989).

The study of tympanic temperature in hot climatic zones in relation to ambient temperature and feeding strategies has so far focused on cattle (Davis *et al.* 2003; Hahn 1999; Mader and Davis 2004; Mader *et al.* 2002). These workers used tympanic temperature to assess the physiological status of the animals.

Drew (1996) compared tympanic membrane temperature and rectal temperature in desert bighorn sheep (*Ovis canadensis nelsoni*) and found that tympanic temperatures were significantly lower (38.4±0.5°C) than mean rectal temperatures (40.9±0.29°C). The author argued that tympanic thermometers provide a safe, non-invasive, and fast temperature measurement that is more responsive to dynamic physiological processes than rectal temperature (Drew 1996).

2.6 Factors affecting feed intake

Terms related to feed intake include: voluntary intake, palatability, acceptability, hunger and appetite. Pond *et al.* (2005) have defined these terms and the many factors involved

in determining feed intake. For this discussion, factors affecting the amount of feed consumed by animals are classified into feed factors, animal factors, environmental factors and feeding management factors (McDonald *et al.* 2002; Pond *et al.* 2005). The effect of climate on digestive function is discussed, as it has an indirect effect on feed intake.

2.6.1 Feed characteristics

Control mechanisms for feed intake operate at the metabolic level and more specifically at the level of the digestive system (McDonald *et al.* 2002). In their review, Forbes and Barrio (1992) argued that intake in the ruminants was controlled by chemo-sensitivity and mechano-sensitivity in the abdomen. The physical form of the feed, the type of carbohydrate and sodium bicarbonate inclusion significantly affect diet selection and feed intake of sheep (Cooper *et al.* 1996; Weston 1966). Mc Donald *et al.* (1990) reported that a mixture of hay and pellets results in higher intakes of sheep in feedlots than in similar sheep offered only pellets.

Metabolic intermediates such as glucose, lipid and other nutrient concentrations in blood signal the start or end of a meal (McDonald *et al.* 2002). Several theories have been established to explain how glucose, amino acid, water and fat affect appetite regulation (Table 2.4). Studies using intravenous infusion of acetate, propionate and butyrate in sheep have shown that short-chain fatty acid concentrations in the blood are feedback signals that regulate feed intake (Anil and Forbes 1980).

Table 2.4 Theories on appetite regulation

Theory	Appetite regulation and reference/s
Glucostatic or chemostatic	A high concentration of glucose in the blood reduces feed intake (Blaxter 1962; Ruckebusch *et al.* 1991)
Amino acid balance	Feed intake is depressed when energy substrates in the blood are more available than amino acids. Conversely the intake of carbohydrate food is lowered when diets are rich in protein (Ruckebusch *et al.* 1991)

Dehydration	Satiety follows dehydration of the digestive tissues and appetite is stimulated by rehydration of the digestive tissues (Ruckebusch *et al.* 1991).
Lipostatic	The hypothalamus modifies the level of DMI and bodily activity in response to changes in body fat (Blaxter 1962).

2.6.2 Animal factors

In ruminants, morphological factors affect feed intake. For example, cattle cannot graze as close to the ground as sheep because of the lack of a cleft upper lip (Hafez 1968b; Klemn 1993). Signals reported to be of importance in several species include: visual inputs and inputs from taste receptors (Fraser and Broom 1990). It has been shown that feed intake and feed preferences of sheep are influenced by odour and taste (Arnold *et al.* 1980). Hinch *et al.* (2004) reported that sheep more readily consumed a new feed if it has odour and flavour characteristics that are familiar to them. DMI of sheep offered rice bran with the odour of dog faeces was much less than DMI of controls (Tien *et al.* 1999). It is likely that the odour of ammonia or other volatile chemicals from excreta might be the reasons for the lower amount of feed intake of sheep.

At the level of the digestive system, signals produced from stretch receptors in the rumen wall that respond to the amount of digesta in the rumen help control food intake (McDonald *et al.* 2002). When the rate of passage out of the reticulo-rumen increases, there is a capacity for an increase of voluntary intake (de Vega and Poppi 1997). The contractions of the stomach generate the subjective sensation of hunger that initiates eating (Blaxter 1962). The time spent by feed in the reticulo-rumen, which depends on the rate of particle size reduction, is also a factor affecting feed intake (de Vega *et al.* 1997; Ruckebusch *et al.* 1991).

The role of the central nervous system, the neuro-transmitters and their roles in controlling feed intake are discussed by Klemn (1993). He suggests there are three groups of neurons located in the hypothalamus, one group located in the lateral and another two groups in the ventromedial areas. Neurons in the lateral area function to promote the drive for eating, while the other two groups register satiety.

The physiological status of the animal is very significant in determining feed intake. Intake is related to metabolic weight ($W^{0.75}$). Feed intake follows, approximately, the changes in metabolic body weight as the animal grows (McDonald *et al.* 2002). Adult animals maintain body weight at the same level while young animals grow rapidly although energy expenditure varies (Hafez 1968a).

An animal's status and age are other factors determining feed intake. A series of experiments with 12 to 14 week old lambs showed that feed intake declined by 73% after two weeks' exposure to ambient temperatures of 32°C (McDowel 1972). Physiological status (pregnancy and fat score) has been shown to limit feed intake (Ketelaars Jan and Tolkamp 1996). In late pregnancy, intake is restricted by the foetus that reduces rumen capacity but intake increases during lactation (McDonald *et al.* 2002). When oestrogen secretion increases during oestrus in ruminants, food intake decreases (Ruckebusch *et al.* 1991). Male fat-tailed Awasi lambs had a significantly higher (1286 g) DMI than females (1148 g) under a neutral (18.7°C) and a high (30.4°C) ambient temperature (Can *et al.* 2004).

2.6.3 Environmental factors

According to the thermostatic theory, feed intake is influenced by the temperature of the body and the environment. Low environmental temperatures cause increases in DMI and high temperatures cause decreases (McDonald *et al.* 2002). Studies in a climate chamber showed that DMI linearly decreases as the ambient temperature increase (Hogan *et al.* 2004).

Heat stress caused by high ambient temperatures stimulates the medial satiety centre and inhibits the lateral appetite centre and DMI is reduced (Gaughan *et al.* 2004). The decrease in DMI reduces heat of fermentation and metabolic heat production enabling the animal to balance heat production and heat dissipation (Holt *et al.* 2004). Li *et al.* (2000) support this observation with their finding that fasting sheep under hot and cool temperatures, with or without water, show lower heat production and rectal temperature. DMI increases in order to increase energy expenditure and heat production and so normal core body temperature (Kraly and Blass 1976).

Higher ambient temperature is the factor responsible for the reduction in DMI. Shearing and live weight changes are other two factors that, when they are associated with higher ambient temperature would affect DMI. Donnelly *et al.* (1974) found that, after

shearing, DMI in sheep in a hot environment increased as much as 10% of their DMI before shearing and the sheep gained weight. Their unshorn counterparts lost weight and had a decrease in DMI of up to 35%. DMI of shorn and unshorn sheep had higher DMI during the night than that during the day. The increase DMI during the coolest hours of the day probably helped the sheep to maintain metabolic heat production to keep them warm (Donnelly *et al.* 1974).

Reductions in feed intake of animals in response to hot conditions can have the unwanted consequence of creating a nutritional imbalance that can lead to lower productivity, higher vulnerability to diseases or, in extreme conditions, death. Studies on live export sheep have shown that higher mortality was related to low feed intakes both pre-embarkation and during the voyage (Norris *et al.* 1989a; Norris *et al.* 1989b; Richards *et al.* 1989).

2.6.4 Feeding management to control feed intake of heat-stressed animals

Manipulating the energy density of the diet, the intake of protein and the timing of feeding can be effective tools for managing ruminant animals in hot climatic conditions. Normally, a decrease of feed intake is expected to occur as an adaptive response to hot-humid conditions. However, decreases in DMI do not always occur. Awassi sheep were found to increase daily feed intake as the lucerne component of a concentrate diet was increased in a warm (27 – 32°C) climate (Bhattacharya and Uwayjan 1975). Bhattacharya and Uwayjan (1975) concluded that the increased intake could be as a compensation to meet the need of energy intake from the lower energy diet. A trial was conducted by Can *et al.* (2004) on fat-tailed male and female Awassi lambs under a neutral (18.7°C) and a high ambient temperature (30.4°C). In this study, high ambient temperature did not significantly reduce the DMI of the lambs (1199 g/d vs 1235 g/d). The similarity of DMI in the neutral and high ambient temperature conditions was thought to be related to digestibility (Bhattacharya and Hussain 1974).

Bailey and Fortune (1992) measured the response of Merino wethers to feed-lotting and the subsequent hot conditions associated with sea transport. They reported the DMI at the end of the feedlot phase was 1 kg/d but DMI declined to about 0.5 kg/d during the first days on the ship, and took almost a week to return to 1 kg/d. The average body-weight loss during the shipping was reported to correlate with this DMI pattern.

Furthermore, the se workers noted that sheep that did not eat well and lost up to 25% of the initial weight at the feedlot were more susceptible to mortality on the ship.

2.6.5 The influence of climate on the digestive function

Generally, an animal exposed to a hot climate reduces its intake of feed in order to reduce heat load to the body (Stewart 1991). Changes in ambient temperature can change the temperature of the rumen (Church 1975). Christopherson (1985) reported a decrease of up to 41% of blood flow into the rumen wall of sheep subject to mild heat stress conditions, and under similar heat-stress conditions, the blood flow to the small and the large intestine decreased by 25% and 3%, respectively (Christopherson 1985). The consequences of this lower blood flow to the gut of heat-stressed animals have been discussed earlier (see betaine Section in 2.11.2).

Rumen motility

Rumen motility in sheep is reduced by a hot environment. The strength of ruminal contractions (mm Hg) after intra-ruminal infusion of warm water in a hot environment (morning 4.6, afternoon 4.5) was significantly lower than that in a thermo-neutral environment (morning 5.6, afternoon 5.0 mm Hg) (Sunagawa *et al.* 2002). Westra and Christopherson (1976) reported wethers kept in warm conditions have a higher mean retention time (38.5 h) than those in the cold (32.5 h). The longer the digesta remain in the rumen the more enzymatic fragmentation takes place, and that in turn increases digestibility (Stevens 1988). DM digestibility of the study was higher (59.9%) than that in the cold (56.6%) (Westra *et al.* 1976). A lower rate of rumen contractions results in longer retention time of digesta and higher feed digestibility.

Rate of passage of digesta

The digesta movement through the gut can be expressed either as a rate of passage or as digesta retention time (McDonald *et al.* 2002). Leibholz (1985) reported that the increase of DM digestibility under heat stress conditions (35°C) was partly explained by the feed longer retention time in the rumen (Leibholz 1985). In contrast, another study showed that ambient temperature (4, 24 and 36°C) had no effect on the rate of passage of both solid and liquid fractions of digesta in sheep offered normal or ammoniated wheat straw (Llamas-Lamas and Combs 1990). These workers found that sheep offered normal or ammoniated straw *ad libitum* had faster passage rates ($P<0.01$) than these with restricted feeding regimes.

Rumination

Studies of the effect of air temperature and humidity on the ingestive behaviour of sheep suggest that rumination activity may be inversely related to environmental temperature (Christopherson 1985; Costa *et al.* 1992). Rumination of Polwarth ewes decreased from 770 s to 25 s as the air temperature increased from 24°C to 45°C (Costa *et al.* 1992). Leibholz (1985) reported that wethers kept in comfortable conditions (18°C) spend a shorter time (9.20 h) for ruminating than those in heat stress conditions (35°C) which spent 10.1 h (Leibholz 1985). The decrease in rumination time of sheep in high ambient temperature could be due to their open-mouth panting. The implication for digestibility is that the longer the time the animal spends ruminating ingesta, the finer the feed particle size is likely to be, and digestibility is therefore likely to be increased (Van Soest 1982).

Feed digestibility

Several studies have found that fibre digestibility is related to ambient temperature. Digestibility of fibre was higher in warm conditions than in cool ones (Bhattacharya *et al.* 1975). For each degree Celsius increase in temperature, diet digestibility increased by 0.2 points (Morand-Fehr and Doreau 2001). The higher digestibility of fibre in hot conditions can be explained by the fact that this component of the feed usually passes from the reticulo-rumen more slowly compared to other dietary constituents or non-fibrous feed components (Allen 1996).

Apparent digestibility of DM and OM was reported to decrease in the rumen when shorn yearling Suffolk wethers were exposed to temperatures of 0 to 2°C (Kelly and Christopherson 1989). Total apparent digestibility of DM and OM was found to be consistently lower in the cold temperature in the same study. A lower apparent digestibility of DM in the cold temperature (0.8°C) has been reported before (Westra *et al.* 1976). The results implied that apparent digestibility of DM and OM would be higher in the hot conditions. However, as discussed earlier, Bhattacharya *et al.* (1974) conducted a series of experiments on sheep and reported that heat stress lowered the digestibility of DM. This inconsistency of results between Kelly (1989) and Westra *et al.* (1976) and Bhattacharya *et al.* (1974) has been explained by Church (1975), who proposed that moderate changes in environmental temperature may have no effect on

digestibility. Christopherson (1985) also suggested that the degree of thermal stress in some studies might not have been large enough to have a significant influence on the animal.

Lowering the ambient temperature did not influence digestibility of nitrogen (Westra *et al.* 1976). Nitrogen digestibility of roughage in cool conditions (53.6 %) was higher than that in hot conditions (48.7 %). Nitrogen digestibility of sheep offered roughage and supplemented with fish meal kept in the hot conditions (74. 9 %) was lower than those kept in cool conditions (77. 8 %) even though nitrogen intake of the two groups was the same (Dixon *et al.* 1999). The higher water intake and urine output in the hot-condition group may explain the phenomenon. Brod *et al.* (1982) reported that when water at temperatures of 0 to 30°C was infused into the rumen, crude protein digestibility of sheep did not change significantly, although the lowest digestion coefficient was recorded for the 0°C water treatment. Christopherson (1985) concluded that exposure to heat has been shown to result in some increases in ruminal ammonia concentration, whereas exposure to cold has caused decreases.

High ambient temperature and low humidity are the climatic factors recorded as affecting DMI, digestibility and therefore DM faecal production of Awasi sheep offered various levels of roughage (Bhattacharya *et al.* 1975). Sheep were kept in normal conditions (21 - 24.1°C and relative humidity 42 – 70.3%) or in high temperature conditions (21 – 32.5°C and 33.3 – 69.3% relative humidity). Mean DMI was significantly ($P < 0.05$) higher (1027 g/d) in sheep maintained under normal conditions than that in hotter conditions (934 g/d). DM digestibility did not differ between sheep in hot conditions (73%) and cooler conditions (70.2%). The significant increase in nutrient digestibility found in this study at higher temperatures was due to a higher crude fibre digestibility (53.6% vs. 45.9%) (Bhattacharya *et al.* 1975).

The effect of temperature on weight gain and feed efficiency

Feed intake, average daily weight gain and feed efficiency are closely correlated. Feed efficiency is the weight gain per unit of feed (Pond *et al.* 2005). When the animals are kept in hot temperatures, they use more energy for heat dissipation and so have less energy for tissue deposition and so feed efficiency is reduced (Church *et al.* 1971).

As discussed above, decreases in digestibility of certain diets, induced by exposing animals to low environmental temperatures, are related to changes in the rate of passage

of digesta through the gastrointestinal tract. Increased rates of passage are due to enhanced gastrointestinal motility and probably, for some diets, to enhanced rumen activity. Changes in these digestive parameters are opposite in response to heat stress (Christopherson and Kennedy 1983). Lambs held under cold conditions ate 4% more feed than the lambs under warm conditions, but had average daily weight gain and feed efficiencies similar to those of the lambs in warm conditions (Von Keyserlingk and Mathison 1993).

In summary, climatic conditions indirectly influence the digestive function of animals and cause changes to the animals' feed and water consumption patterns. A direct effect can be seen in the blood flow to, and the fluid and fibre dynamics of digesta in the digestive tract, and especially in the rumen; this can cause changes in rumen function. Thus, hot climatic conditions can result in reductions in feed digestibility, feed efficiency and average daily gain of animals.

2.7 Feeding behaviour

Feeding behaviour includes several activities, such as seeking and locating feed sources and allocating preferences between them; it describes the way an animal chooses to satisfy its nutritional needs (Hafez 1968a; Klemn 1993; Ruckebusch *et al.* 1991). Changes in feed intake and selection under heat-stress conditions, however, should be considered as an animal's behavioural adaptation to cope with stress (Hafez 1968a). Responses in feeding behaviour can be very complex. This discussion is limited to three factors: the role of the central nervous system, the effect of diurnal pattern of ingestion and the effect of social interactions on feeding behaviour.

2.7.1 The role of the central nervous system in feeding behaviour

Feeding behaviour involves the central nervous system (CNS), hormones, locomotory and sensory capacities and previous experience (Hafez 1968a; Provenza 1995). Feeding behaviour is determined by two antagonistic processes: namely, the initiation of a motivation to eat (hunger) and the cessation of this motivation when the animal is satiated. The stimulus of hunger initiates the search for and intake of food, and satiety stops further eating (Ruckebusch *et al.* 1991). Van Soest (1982) summarised the relationship of feeding behaviour and feed intake and other functional anatomical and physiological factors by means of a diagram (Figure 2.2).

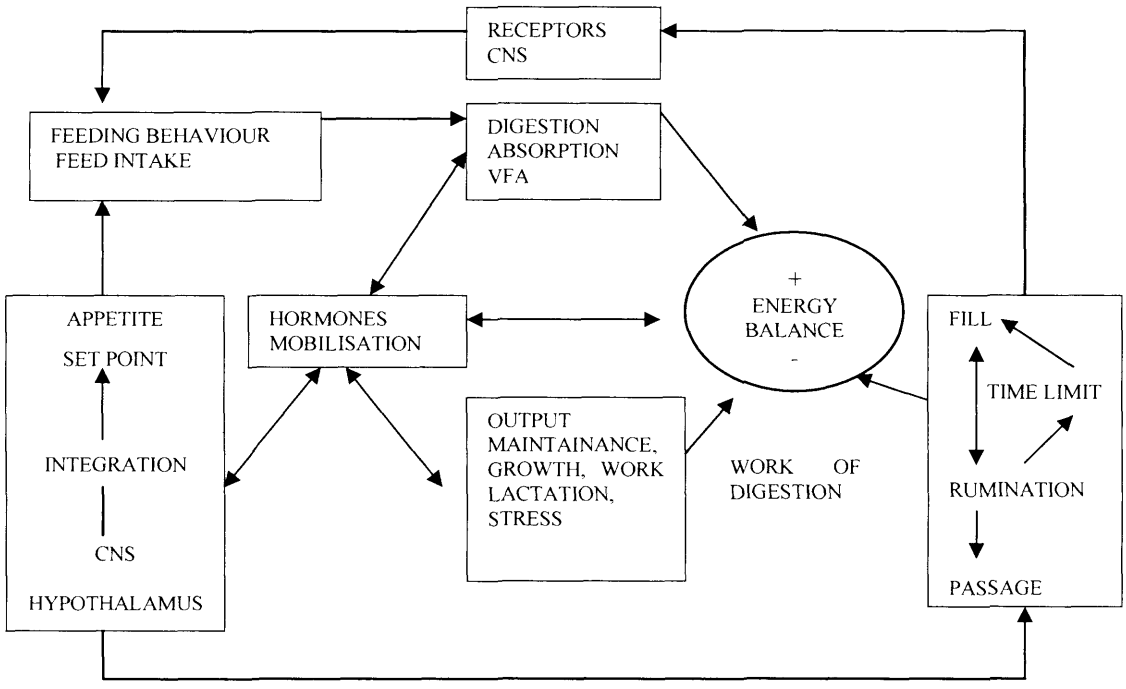

Figure 2.2 A model of the various mechanisms regulating voluntary intake (after Van Soest 1982)

2.7.2 Diurnal patterns in feeding behaviour

Sheep graze 4 to 5 times each 24 h. Intensive grazing begins around dawn and declines at dusk; the longest periods occur in the early morning, depending on the season, and between the late afternoon and dusk (Hafez 1968a). Sheep have been observed to eat intensively between 0800 and 2000 h (Keskin *et al.* 2005). Fraser *et al.* (1990) reported sheep grazed for 10 h. Regardless of time spent on eating and other activities, sheep on average ingested food equivalent to 2 – 5% of their body weight and adults drank as much as 3 – 6 L/d of water (Fraser *et al.* 1990).

Leibholz (1985) noted that diurnal feeding behaviour appears to be affected by ambient temperature. Eight rumen-fistulated wethers were housed at constant ambient temperatures of 18 °C or 35°C. For the sheep in the 35°C temperature, the time spent eating was higher (5.07 vs. 4.93 h), ruminating time was lower (9.20 vs. 10.10 h) and resting time was longer (9.73 vs. 9.07 h) than for the sheep at 18°C. The sheep at 18°C had completed 88% of their 24-hour eating time within 6 h of feeding, while sheep at 35°C had only completed 68% (Leibholz 1985).

2.7.3 Social factors influencing feeding behaviour

Social influences also affect feeding behaviour. Group-fed animals tend to eat more than those in isolation (Table 2.5). When a feed trough was placed in the centre of the yards, sheep in the centre were observed to eat more feed than those near the fence, suggesting feeding behaviour was facilitated by social factors (Mc Donald *et al.* 1990). The negative effect of this social factor was that dominant animals ate more than less dominant animals (Klemn 1993).

Ruckebusch *et al.* 1991) found that the rate of eating was always higher in group-fed sheep whether offered feed *ad libitum* or in restricted amounts. Unfortunately, ambient temperature was not included in the treatments. The study supports the premise that feeding behaviour is strongly influenced by social interactions (Fraser *et al.* 1990). A study by Norris *et al.* (1990), however, found that mortality of sheep from inanition on sea voyages was not due to the competition from other sheep or social dominance.

Table 2.5 Effects of grouping and isolating of sheep on feed intake with *ad libitum* and restricted feeding regimens (Ruckebusch *et al.* 1991)

Item	*Ad libitum*		Restricted	
	Group	Isolated	Group	Isolated
Rate of eating (g/30 min)	265	240	295	285
Time spent eating (h/d)	3.7	5.1	3.9	3.8
Time ruminating (h/d)	5.2	5.3	7.4	7.5
Total intake (kg/d)	1.6	1.6	1.0	1.0

2.8 Factors affecting water intake

As more water is lost at high ambient temperatures, animals need to drink more to maintain their body water balance (Reece 2005). An animal's live weight consists of about 57 to 79% (L/kg x 100) water for various sheep breeds (Yousef and Johnson 1985). The total body water of sheep under dry conditions and higher ambient temperatures is lower than that under wet conditions and at lower ambient temperatures.

There is a decrease in total body water in a hot dry climate that may be an adaptive mechanism for heat dissipation mediated by water; water is evaporated through the skin and via respiration when ambient temperatures increase (Ingram *et al.* 1975; Yousef *et al.* 1985).

The volume of total body water is also affected by age, body fat content, species, availability of drinking water and adaptation to various climatic conditions (McDonald *et al.* 2002; Pond *et al.* 2005; Yousef *et al.* 1985).

2.8.1 Distribution of water in the animal body

Body water can be seen as comprising a cellular component and an extracellular component (Reece 2005; Stewart 1991). About two-thirds of body water is found within the cells (the intracellular fluid), located in skin cells, brain cells and other parts. The extracellular water is located in both the blood plasma and in the spaces between the cells, i.e. the interstitial water (Stewart 1991). All water that is not in cells is considered to be extracellular fluid, comprising the interstitial, intravascular, and transcellular fluid (Reece 2005).

About 15 – 20% of the total body water is held in the rumen (Dahlborn and Holtenius 1990). This rumen water volume benefits animals such as the Bedouin goat which is reported to be able to withstand a period of 3 to 4 d without access to water and then only has a deficit amounting to 25% of its live weight (Olsson *et al.* 1997). However, a higher water content in the rumen might dilute rumen contents, reduce saliva production and decrease rumen motility, leading to a decrease in digestibility of food (Bernabucci *et al.* 1999).

The fluids in the different compartments of the animal body are in dynamic equilibrium with one another and there is a continual interchange of fluid between them (Whittow 1968). Water metabolism is a function of water intake and water loss (Figure 2.3) which will be discussed in the following section.

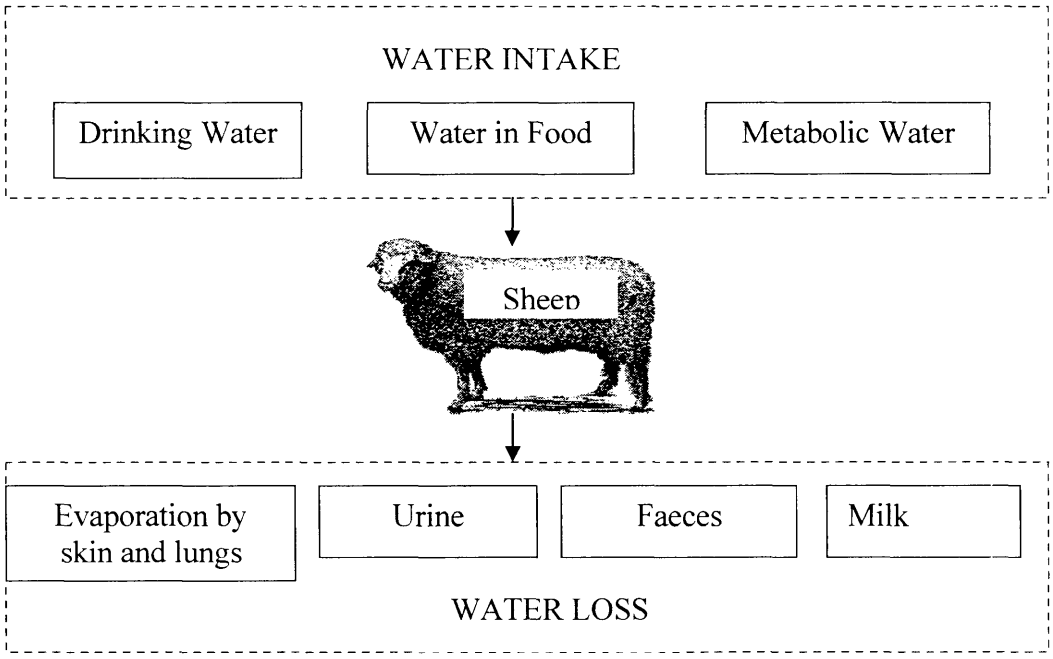

Figure 2.3 Sources of water intake and avenues of water loss (adapted from Yousef *et al.* 1985)

2.8.2 Sources of water intake

Animals derive their water from three sources: drinking water, water contained in feed and metabolic water (McDonald *et al.* 2002; McGregor 2004; Reece 2005; Schmidt-Nielsen 1991; Stewart 1991; Yousef *et al.* 1985). The amount of water drunk is well matched to the requirements of the animal under normal conditions (McDonald *et al.* 2002). The water content of foods can range from 90% in growing plants to over 90 % in some root crops and can be as low as 5% in dry grains (Yousef *et al.* 1985). Metabolic water is derived from chemical reactions in the cell mitochondria. At the end of the electron transfer chain, hydrogen and electrons are combined with oxygen to form water (Reece 2005). The metabolic water yield from 100 g amounts of protein, carbohydrate, and fat is 40, 60, and 110 ml, respectively (Reece 2005).

2.8.3 Avenues of water loss

Evaporation through the skin and lungs and water passed in urine and faeces (Figure 2.3) are all avenues of water loss. Milk is another avenue of water loss as shown by the study of Abdalla *et al.* (1993). Water loss from the body is either an insensible loss or a sensible loss. Insensible losses are associated with vapour losses and occur constantly by evaporation from the skin and by loss of water vapour in exhaled air. Sensible losses

are the visible losses; in the urine, faeces, and body secretions. These losses of water occur continuously (Pond *et al.* 2005), depending upon the mammalian species, its water metabolism and environmental conditions (Stewart 1991).

Water intake is related to water loss (Figure 2.3). The relative importance of each source of water intake and each avenue of water loss is related to many factors, including environmental and dietary factors (Pond *et al.* 2005; Yousef *et al.* 1985). Water temperature is also reported to affect drinking water in cattle (Beede 2005) and sheep (Bailey *et al.* 1962).

2.8.4 Influence of environmental factors on water intake

Water intake increases in a linear way with increasing ambient temperature, either in the field or in a climate chamber. Water consumption of adult Angora goats and adult Merino sheep has been measured when they were grazing on dry unshaded summer pastures in Southern Australia. Average water intake on the hottest days (>33°C) for both goats and sheep was twice their water intake at 25°C (McGregor 1986). Sheep kept under controlled room temperatures of 20, 25, 30 and 35°C had water intakes of 3.2, 3.1, 3.6 and 4 L/d, respectively (Hogan *et al.* 2004).

Water intakes of different species of sheep in different physiological states have been studied under heat-stress conditions (Abdalla *et al.* 1993; Salah *et al.* 1995; Stephenson *et al.* 1980). Heat-stressed ewes had increased water intake (27%) and increased plasma prolactin concentration (22%), but their daily urine output (27 – 36 ml/kg body weight) was unaffected (Stephenson *et al.* 1980). Water intake of crossbreed pregnant ewes at 21-d prepartal was 6.4 L/d and 12.8 L/d when ambient temperatures were 20°C and 35°C, respectively. These intakes had increased to 9.5 L/d and 16.9 L/d 42 d after lambing (Abdalla *et al.* 1993). The researchers concluded that the higher water intake of heat-stressed ewes during pregnancy was probably a function of their greater need for cooling mechanisms, but the increase water intake after parturition was possibly for milk production as well as cooling. (Abdalla *et al.* 1993) concluded that lactation in sheep is a more powerful stress stimulus than pregnancy under either thermo-neutral or hyperthermic conditions. Salah *et al.* (1995) studied the effect of inhibiting prolactin secretion on thermo-regulation, and water and food intake in heat-stressed fat-tailed male lambs. The heat-stressed lambs, given bromocriptine to lower prolactin secretion,

did not increase their water intake. Mean water intake was not affected by the bromocriptine treatment, while DMI decreased significantly.

The higher water intake of sheep in higher ambient temperatures can be explained by the increased evaporative loss of water which is an important mechanism for regulating body temperature (Church 1982). The animals drink more water in hot environments to compensate for water loss (Beede 2005).

2.8.5 Influence of dietary factors on water intake

Diet affects water intake because feed and water are mixed together as they are ingested and pass through the digestive tract of the animal. The saliva and other gastric juices start to flow during eating which explains why ruminants drink mostly in connection with feeding (Olsson *et al.* 1997; Wilson and Tribe 1963). Diet factors affecting the quantity of water intake are the type of feed (such as whether it is concentrate, fresh forage, fermented forage, or hay) and the nutrient composition of the mixed feed (DM, sodium and crude protein) (Beede 2005).

Dry matter content

DM intake by ruminants is highly correlated with water intake (McGregor 2004). For example, non-reproducing sheep were reported to drink 2 L of water for each kg DMI (Church 1982). He showed that a lamb weighing 45 kg and ingesting 1.5 to 2 kg of feed might be expected to drink 3 to 6 L of water (Church 1982).

The DM content of the diet influences drinking water intake as water intake in feed reduces the animal's need to drink. When forage with a high water content is very abundant, less water is drunk (Pond *et al.* 2005). For pasture grazing, the estimated water requirement for sheep is based on the pasture conditions. For example, the water requirement suggested for mature sheep on dry pasture is 7 L/d higher than that for mature sheep on green pasture (3.5 L/d) (Abacus 2004; ANZECC 2000).

Sodium content

There is sufficient evidence that the intake of sodium chloride or other salts greatly increases the intake and excretion of water (Godwin and Williams 1986; Wilson 1966). When sheep were infused intraruminally with 0 to 2000 mmol/d sodium chloride, their water intake increased from 1.09 to 6.36 L/d (Godwin *et al.* 1986). The mode of action of sodium in influencing water intake and water flux in the intercellular space has been

explained (Ruckebusch *et al.* 1991). Animals will drink after eating because of signals from the central osmoregulatory mechanisms that include, among other factors, increased sodium excretion (Mathai *et al.* 2001).

Protein content

High levels of protein also increase water intake because higher urinary outputs are needed to excrete the urea formed in response to deamination of excess amino acids. A study was conducted using desert rams kept in unshaded and shaded environments and offered a diet of concentrate and lucerne hay (Abdelatif and Ahmed 1992). These workers reported a higher water intake (L/kg DMI) with lucerne hay. Fish meal rich in protein was reported to cause increased water intake of sheep in both cool and hot room conditions (Dixon *et al.* 1999). When urea is used as the major source of nitrogen in ruminant diets, a urine volume increases (Pond *et al.* 2005), causing drinking water to increase.

2.8.6 Influence of water temperature on water intake

Studies on the effect of water temperature on water intake have focused on cattle, with particular emphasise on dairy cows (Ittner *et al.* 1951; Lofgreen *et al.* 1975; Looper and Waldner 2002; Osborne *et al.* 2002). Beede (2005) reviewed several studies on the effect of temperature of drinking for dairy cows. The cows preferred warmer water during summer; however, the core finding of this review was that chilled water seemed to effectively reduce the heat load of the animal, presumably because cool drinking water had a direct effect, cooling the reticulum as well as reducing the thermal load (Beede *et al.* 1986; Blackshaw *et al.* 1994).

The effect of water temperature has been investigated in goats. One study focused on the goats' preference for warm drinking water which induced hyperhydration in heat-stressed lactating goats (Olsson and Hydbring 1996). Two rooms were used, i.e. a normal (18 – 19°C) and a hot room (39 – 40°C). Goats in each room were offered a choice of drinking water with temperatures of either 15°C or 35°C. The goats in the normal room drank more warm water (6 L/d) than cold water (1.7 L/d). The goats in the hot conditions drank 11.5 L/d of the warm water but only 2.0 L/d of the cold water (Olsson *et al.* 1996). These workers concluded that goats prefer to drink warm water when given a choice between water at temperatures of 35 and 15°C. Olsson *et al.* (1997) also observed the drinking behaviour of goats deprived of feed and offered water

at different temperatures. They suggested that the effect of temperature of the drinking water must be considered with factors such as lack of DMI and hyponatraemia in order to explain the goats' reluctance to drink during food deprivation (Olsson *et al.* 1997).

In a study of sheep in a cold chamber (-12°C), water intake tended to increase (from 0.72 L/d to 0.84 L/d) when water temperature was raised from 0°C to 30°C (Bailey *et al.* 1962). In the same study the sheep that drank water at 30°C had a rectal temperature of 38.7°C which, rather unexpectedly, was significantly ($P < 0.05$) lower than for those that drank water at 0°C or 10°C but had higher rectal temperatures of 39.0°C. A question remaining to be answered is whether the sheep in the cold conditions which drank more hot water were doing this to increase their core body temperature.

2.9 Urine production

The urinary system is responsible for the excretion of many waste products from the body. It is also important for maintenance of homeostasis. The functions of the urinary system include: the regulation of water balance, pH, osmotic pressure, electrolyte levels, and concentration of many plasma substances (Frandson 1981). Urine also functions as the main route of excretion of nitrogenous and sulphurous metabolites of body tissues and for some of the mineral elements (Church 1975).

Urine accounts for a small fraction (10 – 15%) of total water loss in sheep, even when water is freely available (Maloiy *et al.* 1971). The normal excretion of urine for a sheep weighing 40 – 50 kg is 1 – 2 L/d. Urine volume varies each day, depending on environmental factors, water intake, feed intake, rate of respiration, and the effect of hormones on the kidney (Church 1975). These factors cannot be assessed individually when urine production needs to be measured. That is because again urine volume is one part of water gain and water loss (Figure 2.3).

Sheep in a hot environment (32 – 40°C) drink significantly ($P<0.01$) more water 5.6 L/d and excreted more ($P<0.01$) urine (2.8 L/d) than sheep in a cool environment (13 – 15°C) that drank 2.5 L/d of water and excreted 1.1 L/d of urine (Dixon *et al.* 1999). Sudanese desert sheep in the shade were found to excrete more urine than those exposed to the sun (Abdelatif *et al.* 1992). Under high temperature conditions, it appears that urine output was more affected by the amount of water drunk than the higher ambient temperature (Church 1975). Guerrini *et al.* (1982) found the pattern of

urine production following the pattern of water intake under all climatic conditions. Sheep that excreted more urine were those that drank more water.

Even though urine volume was higher in hot conditions, Malloy *et al.* (1971) found daily heat load had no significant effect on urine volume in fat-tailed sheep kept at an ambient temperature of 22 °C or 22 – 40°C and offered either *ad libitum* or restricted water. An interesting finding in a study in different climatic conditions was that the highest urine production occurred in cool-humid conditions, although the water intake of the sheep in this group was lower than in hot-humid and hot-dry conditions (Guerrini *et al.* 1982). The higher loss of water via respiration might explain the findings of Malloy *et al.* (1971) and (Guerrini *et al.* 1982). Similar reasoning explains why the urine output of sheep was reduced by 0.5 L/d when they were subjected to hot conditions (Macfarlane *et al.* 1966).

Feed and nutrient intake affect urine production (Section 2.8.5). Ahmed *et al.* (2004) reported urine excretion was significantly higher in sheep offered lucerne hay than in those offered grass hay. He suggested this was due to the higher crude protein concentration in the lucerne hay that generated higher nitrogenous losses through urine (Ahmed *et al.* 2004). Urine volume was reported to be higher in a group of Sudanese desert sheep offered concentrate than in those offered lucerne (Abdelatif *et al.* 1992).

The volume of urine produced by an animal depends on various interrelated factors: climatic conditions, drinking behaviour, physiological functions, and feed intake. Sheep under hot conditions, to maintain a homeostasis, drink a lot of water that is used for evaporative cooling. This requirement is additional to the requirement for water to maintain kidney excretion of minerals and products of metabolism.

2.10 Faecal output

The amount of faecal DM excreted per day is affected by a number of variables; the two most important are the amount of feed consumed and the digestibility of the feed (Church 1975). For example, sheep in hot conditions are generally reported to excrete less faeces than those in cool conditions as the consequence of their lower feed intake (Dixon *et al.* 1999) and possibly also a higher digestibility (see Section 2.6). Dixon *et al.* (1999) found that sheep in hot room, when offered roughage and fishmeal, had higher DMI but excreted less faecal DM than their counterparts in the hot room offered

only roughage, i.e. feed intake 1030 vs 989 g/d and faecal DM output 382 vs 438 g/d. This was because DM digestibility of the diet containing fishmeal was higher than that of the roughage alone (62.9% vs 55.7% g) (Dixon *et al.* 1999).

The water content of faeces from sheep ranges from 55% to 70% depending upon the animal's diet and the capability of the species to form faecal pellets by absorbing more water from the large intestine (Church 1975). Macfarlane (1968) reported that sheep on dry summer pasture produced faecal pellets containing about 60% water, but after 5 d without water, the pellets were drier at only 45% water. The dry faeces in the dehydrated sheep is because of re-absorption of more water from the gastro-intestinal tract in response to the higher osmotic pressures in blood of the dehydrated sheep (Olsson *et al.* 1997).

Because faeces and urine leave the animal at body temperature, urine and faeces could be considered to be a way of losing heat (Currie 1988; Reece 2005); however, the heat loss through excretion of faeces and urine is considered negligible.

2.11 Feeding management options for animals in hot climates

2.11.1 Altering feed formulation

Some diets may be less stressful for animals subject to hot conditions than others. The objectives of changes to the diet are to maintain nutrient intake, re-establish homeostasis, improve rumen ecological conditions and microbial activity and increase the efficiency of absorption and metabolism of nutrients. However, there is no single diet formulation that can satisfy all these objectives. Changes in protein and energy content of the diet are the most likely to be beneficial.

Altering protein content of diets

Altering the protein content of diets for heat-stressed animals is important because of the relationship between energy and protein. When energy is limited, protein is catabolised and serves as an energy source (NRC 1981). The direct effect of climate on energy requirements has a consequential effect on the protein required for growth or production. It has been suggested that, when formulating diets with respect to the thermal environment, each nutrient, whether protein or energy, should be included separately to meet the requirements of the animal (Conrad 1985; NRC 1981). When feedlot animals are exposed to hot conditions, average daily gain (ADG) is lowered

because of a lower DMI, but also possibly because the gross efficiency of conversion of nutrients to tissue is reduced (Ames *et al.* 1980). Protein efficiency ratios, for example, were altered at ambient temperature between 10 and 20°C which corresponded to the maximum daily weight gain and the most efficient use of feed ingested (Ames and Brink 1977; Ames *et al.* 1980; Conrad 1985; NRC 1981). Because ADG is decreased and protein efficiency ratio is lowered during heat stress, Ames and Brick (1977) hypothesised there would need to be an alteration in the amount of available protein to accommodate the thermal environment and protein efficiency ratio of growing animals.

Altering the energy content of rations

Several studies have been conducted to find an appropriate diet formulation to reduce the effect of environmental and metabolic heat loads (Bhattacharya *et al.* 1974; Dixon *et al.* 1999; NRC 1981). Low concentrate diets (35%) have been reported to have lower heat increments than higher concentrate diets (70%) when fed to lambs (Bhattacharya *et al.* 1974). In contrast, it has been reported that diets containing higher percentages of roughage have the higher heat increment (NRC 1981). Molar proportions of acetate: propionate:butyrate which were 70:20:10 for roughage diets and 60:30:10 for concentrate diets may help explain the difference of heat increment produced (Nolan *et al.* 1986).

To reduce the heat energy provided by roughage diets, formulations with different levels of fibre and fat have been suggested (Bhattacharya *et al.* 1974). The most important finding regarding feeding management was that digestibility depressions were most severe when diets contained 75% roughage and the addition of fat seemed to alleviate the depression (Bhattacharya *et al.* 1974). Including fat in diets maximises energy intake and has a minimal effect on rumen heat production compared to diets providing a similar energy intake from carbohydrates; this is because the major components of fat, long fatty acids, are not fermented in the rumen (Beede *et al.* 1986; Bhattacharya *et al.* 1974).

2.11.2 Altering feeding times

Animals alter their times of feeding and levels of feeding activity in response to changes in the environment and metabolic heat. Because heat production increases during and after feeding, Brosh *et al.* (1998) suggested that livestock should be fed in the cooler hours of the day, allowing easier non-evaporative heat loss from the body to

the environment. In the summer months, when the ambient temperature is higher during the day, restricting feed intake in the morning in order to reduce metabolic heat production is a rational feeding strategy (Murphy *et al.* 1994). Davis *et al.* (2003) suggested a strategy of switching feeding time from morning to afternoon and found this strategy resulted in a reduction in mean daily body temperature. Shevi *et al.* (2001) also concluded that supplying feed in the afternoon reduced heat load during the warmest hours of the day and so minimised the effect of thermal stress on immune function and udder health of Comisana ewes.

Restricted feeding is a management practice that may help farmers to achieve an increase in production efficiency while reducing production costs (Hermesmeyer *et al.* 2002). A restricted intake level in growing lambs has a small effect on ruminal pH as well as ammonia concentration (Hart and Glimp 1991).

2.11.3 The use of feed additives for heat stress management

Feed additives are defined as feed ingredients of a non-nutritive nature that stimulate production, improve the efficiency of feed utilisation, or are beneficial to the health or metabolism of the animal (Pond *et al.* 2005). The use of feed additives aims to improve performance in farm animals by optimising the functions of the digestive flora (Fonty *et al.* 1993). Common feed additives include antimicrobial agents, e.g. antibiotics, antibacterial agents, and antifungal agents (Pond *et al.* 2005). Antibiotics are used to improve the health and well-being of animals and also for growth promotion. Organic acids, probiotics, prebiotics and feed enzymes are used as alternatives to antibiotics whose use may lead to the development of resistant bacteria (Choct 2001).

Interest in feed additives has also been directed to the possible use of feed-intake stimulants as a way of increasing feed intake in heat stressed animals. Elfazepam was found to increase hunger drive, feed intake and the acceptance of feed (Baile and McLaughlin 1979). In contrast, penicillin or erythromycin (Hogan and Weston 1969) and monensin have been reported to reduce feed intake.

Betaine

Betaine is another potential feed additive. Betaine ($(CH_3)_3NCH_2COOH$) or the trimethylglycine cation is an organic compound with three methyl groups (Cronje 2005). Betaine is able to support or even replace methionine as a methyl donor in physiologically important body processes (Fernandez *et al.* 2004). Immune function is

dependent on the availability of sufficient methionine (Fernandez *et al.* 2004). Methylation reactions are especially important in stressed or diseased animals to build immune defence mechanisms and support the synthesis of polyamides that play a role in repairing tissue.

Tucker *et al.* (2006) consider the two roles of betaine in animals are as an osmo-regulant and a co-factor in metabolic processes. Betaine plays a role in stabilisation of water balance, enzyme function and membrane integrity during osmotic stress (Augustine *et al.* 1997; Fetterer *et al.* 2003). Betaine also has osmo-protective properties that are essential to maintain the nervous, immune, renal and cardiovascular systems.

Tucker *et al.* (2006) stated that betaine also functions to maintain the integrity of the gut wall and to reduce dehydration of cells. In heat-stressed animals the blood supply to the gut is reduced while it is redistributed to the skin (Christopherson 1985). This can damage cells lining the gut and endotoxins can enter the body. Endotoxins cause tissue damage and an acute phase immune response (Cronje 2005; Sakurada and Hales 1998). Betaine, an amino acid that is found naturally in the cells of micro-organisms, plants and animals, is believed to repair this tissue damage and protect against the effects of endotoxins (Cronje 2005; Fetterer *et al.* 2003; Kidd *et al.* 1997). When given to heat-stressed animals, betaine is believed to not only reduce tissue damage but also to increase resilience to heat (Cronje 2005).

Physiologically, betaine is one of several compounds used by cells to regulate osmotic pressure (Kidd *et al.* 1997). Chickens fed a diet containing betaine showed increased body weight and feed conversion efficiency, lower wound score and mortality and increased resistance to parasites, via both acquisition of infestation and parasite development. The increased body weight and feed conversion efficiency can be explained by the effect of betaine on nutrient absorption; betaine alters the viscosity, thickness or chemical composition of mucus covering the epithelial surface (Kettunen *et al.* 2001).

Probiotics

Probiotics, such as live yeast cultures, are another recent feed additive for animals. The concept of probiotics is to use cultures of live organisms to replace harmful microbes with a healthy ones in the gut (Choct 2001). Probiotics may be added to the diet in order

to control intestinal infections in animals and improve nutrient utilisation (Pond *et al.* 2005). They have been found to improve feed intake and stabilise the intestinal flora as well as stimulate bacterial activity in the rumen, stabilise rumen pH, increase propionate and reduce acetate proportions in the total VFA, and reduce methane and ammonia production (McDonald *et al.* 2002).

The rumen has a complex microbial ecosystem that can be manipulated by using appropriate probiotics to achieve better productivity. The most commonly used group of probiotics are lactobacilli with *Bifidobacterium*, *Propionibacterium*, *Enterococcus* and *Bacillus* spp (Kamra and Pathak 2005). The beneficial effects of a balanced gut microflora have been described and include increasing feed digestibility and enhancement of the immune system (Choct 2001).

Several studies have demonstrated that probiotics improve thermoregulatory function and enhance animal productivity. When 3 - 5 g/d of fungal cultures were added into the diet of dairy cattle, their body temperatures and respiration rates decreased in hot weather (Huber *et al.* 1994). It was found that a probiotic, based on an anaerobic rumen fungus (*Neocallimastix sp.*), increased intake and live-weight gain in calves following weaning (Theodorou *et al.* 1990). Abd El-Ghani (2004) concluded yeast culture supplementation of the diet of lactating Zaraibi goats had a beneficial effect on productive performance, and the daily inclusion of up to 6 g of yeast culture in lactating Zaraibi goats diets was recommended under field conditions. Yeast supplementation increased the number of rumen cellulolytic bacteria and improved feed digestibility. Stimulating rumen microbes by the use of probiotics can improve digestion and consequently increase feed intake (Fonty *et al.* 1993). Cows ingesting yeast culture had higher concentrations of total anaerobic and cellulolytic bacteria in the rumen (Harrison *et al.* 1988). Wiedmeier *et al.* (1987) used a mixture of yeast culture and other products and found that the addition of *Aspergillus oryzae* and a yeast culture to the diets of cattle increased fibre digestibility. The researchers considered that this combination probably provided stimulated rumen bacteria and in addition provided cellulases that increased fibre digestibility.

Other workers have not obtained benefits from the inclusion of yeast cultures in diets. For example, Chademana and Offer (1990) reported that rumen pH, rumen liquid outflow rate, rumen ammonia concentration, total volatile fatty acids concentration, and

molar proportions of acetate, propionate and butyrate were not affected by the inclusion of yeast culture and digestibilities of OM, neutral detergent fibre and gross energy were also unaffected.

Plant extract and yucca

Extracts from the plant *Yucca schidigera* contain urease inhibitors that appear to reduce tissue ammonia in animals and may protect against ammonia toxicity (Pond *et al.* 2005). A study of the effects of *Yucca shidigera* extract on ruminal ammonia concentrations and micro-organisms has also shown that saponins from the extract can suppress ciliate protozoa. The benefit for the host is the microbial yield in the rumen is improved (Wallace *et al.* 1994). Wallace (2002) emphasised the roles of saponins and essential oil secondary plant compounds that have been used to manipulate the rumen. Long and Jie (2005) studied the effect of *Yucca schidigera* extract on the rumen micro-organisms of 12 adult male sheep. The sheep were divided into 4 groups and administrated sarsa-saponin (a *Yucca schidigera* extract) at 0, 100, 200 and 300 mg/kgW, respectively. The protozoa numbers in the rumens of the group given 300 mg/kgW were lower ($P<0.05$) than those of the control and the 100 mg/kgW groups, but similar that of the 200 mg/kgW group (Long and Jie 2005). In a study by Santoso *et al.* (2004), sheep given *Yucca schidigera* had the lowest rumen ammonia concentration (Santoso *et al.* 2004).

Feed enzymes

Enzymes have been isolated from every type of living organism (McDonald *et al.* 2002). Ruminants themselves do not produce fibre-degrading enzymes, but they have bacteria, fungi, and protozoa in their rumen that can break down fibre. The host provides the micro-organisms with a suitable growth habitat and as well as releasing the energy from fibrous feed, the microbes supply protein, vitamins, and short-chain organic acids to the host (Russel and Rychlik 2001). Improvements in animal performance through enzyme supplementation can be attributed mainly to improvements in ruminal fibre digestion, resulting in increased digestible energy intake (McDonald *et al.* 2002). Wiedmeier *et al.* (1987) argued that *Aspergillus oryzae* produces enzymes that break down several parts of fibrous structure of feed and it aids rumen cellulolytic bacteria in the complete depolymerisation of cellulosic material into simple sugars.

Fungal cultures and heat stress cows has been reviewed. Huber (1994) found that rectal temperature and respiration rate of cows fed Aspergillus Oryzae Extract (AOE) were lower than control (Table 6). Rectal temperatures of control were 39.2°C, 39.1°C, 39.3°C and 38.8°C while those of their counterparts that fed AOE were 39.0°C, 39.2°C, 39.3°C and 38.7°C. Huber (1994) at the same review also found that respiration rates of cows fed AOE were 63 bpm, 82 bpm, 74 bpm and 52 bpm lower than those of control 67 bpm, 79 bpm, 70 bpm and 51 bpm, respectively.

2.12 Summary of literature review

Sheep modify their physiological and behavioural characteristics through adaptation when exposed to high ambient temperatures or hyperthermic conditions. The modified physiological characteristics include an increase in respiration rate and in skin, rectal and tympanic temperature. Behavioural adaptations include reduction in DMI and increase in water consumption and seeking of shade.

Higher ambient temperature depresses feed intake. During the hottest hours of the day when the energy and nutrient requirement for thermoregulation and maintenance are increased and feed intake is depressed, requirements may not be met. Many factors are responsible for the decrease of DMI in heat-stressed sheep but there are also feeding management strategies available that can assist the animal to overcome the detrimental effects of low nutrient intake. The efficiencies of digestion (where rumen physiology plays a major role), and of absorption and excretion are important because of the limited amount of ingested feed. The use of certain feed additives, such as probiotics, plant extracts and enzymes can increase feed efficiency. However, there is little information on whether these feed additives help alleviate heat-stress effects in ruminant animals.

Similarly, there are few studies on the effects of drinking water temperatures on intake of water and on heat stress and body temperature in ruminants. One study on Black Bedouin goats (Jessen *et al.* 1998) recorded the effect of water temperature on brain and blood temperature but there is almost no information on the effect of drinking water temperature in heat-stressed sheep.

The experiments reported in this thesis focus on the potential of a yeast-based feed additive as a means of alleviating the detrimental effects of heat stress in Merino sheep, and the effects of, and preference for, drinking water of different temperatures in sheep.

Effects of these treatments on the physiology of the sheep under hot climatic conditions were also recorded.

Chapter 3 General Materials and Methods

This study reported in this thesis was conducted as two related experiments. General materials and methods used for both experiments are described in this section. Specific materials and methods are discussed in Chapters 4 and 5. The Animal Ethics Committee of the University of New England approved the use of the animals in these studies with authority numbers AEC 05/118 and AEC 06/124.

3.1 Animals, feed and water

A group of 22 Merino wethers (one-year old) raised in the 'Trevenna' paddock at the University of New England, Australia from July 2005 to November 2005 formed the flock from which experimental animals were drawn. Sixteen sheep (live weight 40.4 ± 4.53 kg) were selected for Expt 1. They were returned to the paddock at the conclusion of Expt 1 (22 November 2005). In August 2006, 8 sheep (47.01 ± 3.5 kg) from the same flock had rumen cannulae inserted surgically in preparation for Expt 2. Individual metabolism crates were positioned in 2 rooms with water and feed troughs fitted. A separator was fitted under each crate to direct urine and faeces into different receptacles.

Lucerne (*Medicago sativa*) pellets that are also used in the live transportation of sheep to the Middle East were offered as the experimental diet for Expt 1. Lucerne chaff was offered to the sheep in Expt 2. Rations were offered to the sheep *ad libitum*. The nutrient composition of the diets is presented in Tables 3.1 and 3.2. Clean tap water was always available in individual water troughs. Water was added to each trough manually in Expt 1, and was pumped and circulated automatically from a 20-litre plastic container in Expt 2.

In Expt 1, water temperature was recorded twice daily just before 0900 h and just before 1700 h. Measurements were taken using a glass thermometer with a scale ranging from -10 to 110°C. In Exp 2, water temperature was a treatment and was measured with a thermometer placed in each individual water trough.

Table 3.1 Nutrient content of lucerne pellet and yeast-based feed additive used in Experiment 1

Nutrient content (DM basis)	Lucerne pellet	Yeast-based feed additive
Dry matter (g/kg)[1]	879	-
Organic matter (g/kg)[1]	925	-
Crude protein (g/kg)[1.2]	131	450
Moisture (%)[1.2]	12.1	7
Mineral ash (%)[1.2]	7.5	6
Fat (%)[2]	-	1.2
Potassium (%)[2]	-	1.8
Thiamine (ppm)[2]	-	13
Riboflavin (ppm)[2]	-	6.5
Nicotinic Acid (ppm)[2]	-	5
Pantothenic Acid (ppm)[2]	-	12
Pyridoxine (ppm)[2]	-	2.8

[1]Feed analysis was conducted in the laboratory of Ruminant Nutrition at the University of New England

[2] Nutrient composition as written on the commercial container.

3.2 Climate chambers

Two climate laboratory rooms in the animal house of the University of New England, designated 'cool' and 'hot' rooms were used. Each room was 4 m x 4 m and was heated with a hot water pipes. Cool water could also be pumped through the system as required. A thermostat controlled the desired climatic conditions via a Honeywell

computer system that opened and closed valves controlling the amount of water, either cool or hot, to be pumped. The wall of the room was covered by metal.

Table 3.2 Nutrient composition of the lucerne chaff used in Experiment 2*

Nutrient (% DM basis)	Period 1	Period 2	Period 3	Period 4
Dry matter	87.2	90.55	89.46	91.22
Organic matter	91.5	91.00	90.80	90.80
Ash	8.48	9.00	9.20	9.20
Nitrogen	2.80	2.95	3.40	3.41
Crude protein	17.5	18.44	21.25	21.31

* Values are determined in the laboratory of Ruminant Nutrition, the University of New England

In the cool room, ambient temperature was set at 18°C and, in the hot room, at 40°C between 0800 and 1700 h and 30°C from 1700 to 0800 h. Ambient temperature and relative humidity in the two rooms were controlled by a thermostat and humidistat. Relative humidity in the two climate chambers was maintained at 60%. The temperature in the hot room was increased gradually during the adaptation period. The rooms were illuminated with electric lights during the day from 0800 h to 2000 h.

Ambient temperature, relative humidity and water temperature were measured manually in both room throughout the experiments. Room temperature and relative humidity were recorded four times daily in several locations within each room to ensure that climatic conditions were consistent within each room. Relative humidity was recorded according to the conversion table based on dry-wet bulb recording.

In Expt 2, ambient temperature and relative humidity were measured automatically using a Honeywell computer system.

3.3 Body temperature measurement

Body temperature measurements undertaken in these studies included wool, skin, ear and rectal temperature. Wool was measured from parts of the wither, mid-side and

head, and skin was measured from parts of both wither and mid-side. In Expt 1, wool, skin and ear measurements were made twice a day at 0900h and 1700 h. Infrared thermometers were used to measure wool, skin and ear temperature.

In Expt 2, rectal temperature was measured using a calibrated thermistor connected to a computer for automatic recording. A rectal probe was inserted 7 cm into the rectum and temperature was recorded every second, 24 hours daily.

3.4 Respiration rate measurement

In Expt 1, respiration rate was calculated by counting 30 flank movements when the animal was standing; the time measured in seconds on a stop watch was converted to breaths/min (bpm). Duplicates estimates of respiration rate were made at 0900 h and 1700 h.

In Expt 2, respiration rate in the cool room was measured manually by counting flank movements while that in the hot room was measured by a pressure transducer (C. Palmer, England) combined with a strap encircling the body of the sheep at the level of the chest. Respiration rate, recorded following body temperature measurement, was then converted to a panting score (Table 3.3).

Table 3.3 Panting score assigned for sheep (adopted from Silanikove 2000)

Respiration rate (bpm)	Panting score
40-60	Low
60-80	Medium high
80-120	High
Above 200	Severe heat stress

3.5 Measurement of feed intake and water consumption

In these experiments, total daily feed intake was calculated by subtracting the feed remaining in the morning from the total feed offered the previous day. Feed offered and

feed refusals were sampled daily and DM content was determined to allow calculation of daily DMI.

Feed offered and refusals were weighed in Expt 1 with an electrical scale ("AND" electrical balance-EP-40 KA – max 40 kg). In Expt 2, feed offered and refusals were weighed with a digital balance, "Bonso" model-322, capacity 2000 g, graduation: 1 g.

In Expt 1, the clean tap water used to fill the drinking troughs was measured with a 2000 ml plastic cylinder. Water intake was corrected for evaporation rate in each room. Evaporation rate was determined by subtracting, from the amount offered, that remaining the next day before fresh feed and water were offered. In Expt 2, the amount of water offered and refused was weighed to determine the amount drunk (after correction for evaporated water as detailed in Chapter 5).

3.6 Measurement of faeces and urine

Urine/faeces separators placed under each metabolism crate were used to direct faeces and urine into separate containers. Faeces were weighed with an electrical scale ("AND" electrical balance-EP-40 KA – max 40 kg) and 10% of the daily output was sub-sampled and stored at 4°C before thawing and grinding. A description of the laboratory procedure for DM calculations is given in Section 3.8.

The volume of urine excreted (ml/d) was measured with a scaled plastic tube. In Expt 1 only urine volume was measured. In Expt 2, urine volume, urine pH and urine nitrogen excretion were measured.

3.7 Live weight measurement

Live weight was recorded by weighing animals using a portable electrical scale (Ruddweig, Km-1 electronic weighing system, New Zealand) on the first and the last day of each experimental period. No curfew period from feed or water was imposed prior to weighing.

3.8 Dry matter content and digestibility

Dry matter samples of feed and faeces were determined after two periods of oven drying, at 60°C and 105°C respectively. In Expt 1, the stored feed and faecal samples were thawed, individually mixed and sub-sampled at 300 g. The samples were put into aluminium trays, then oven-dried to a constant weight at 60°C for 48 h and DM 1 was

determined. In Expt 2, feed and faeces samples were dried daily at 60°C for 48 h. After this step, analytical procedures were the same for both experiments.

Samples were ground to pass a 1 mm sieve and DM2 was determined on duplicate samples of 2.5 - 3 g. For the DM2 determination, samples were placed in labelled crucibles then dried in an oven at 105°C overnight. The calculations were as follows:

Percentage DM1= [[(g aluminium tray + g sample before oven) – (g aluminium tray + sample after oven)] / (g aluminium tray + samples before oven)] x 100.

Percentage DM2 = [[(g crucibles + samples before oven) – (g crucible + samples after oven)] / (g crucibles + samples before oven)] x 100.

Percentage DM 3 (DM3) = %DM1 x %DM2.

Percentage moisture = 100 - % DM3.

After determining DM2, samples were placed into a carbolite furnace with 3216 PI temperature controller to be slowly burned at 350°C for 1 h and then at 600°C for 2 h to become ash. This adjustable controller temperature program allows for one of a number of methods which are suitable for dry ashing as recommended by AOAC (Horwitz 2002). After cooling, samples were weighed and then OM and ash were calculated.

Percentage ash = [[(g crucible + g sample before furnace) – (g crucible + g sample after furnace)] : (g crucible + sample before furnace)] x 100.

Percentage OM = (100%) – (% ash).

Nutrient digestibility was assessed by determining DM, OM and nitrogen. DM and OM digestibility was determined in both experiments, while nitrogen digestibility was determined only in Expt 2. In this study, the conventional method of digestibility was used.

Apparent digestibility (%) = [[(g nutrient intake – g nutrient in faeces)/ g nutrient intake]] x 100

DM digestibility = [[(% DM3 x feed offered) + (%DM3 X feed refused) / 2] – (%DM3 faeces)] x 100

OM digestibility = [[(%OM x %DM3 feed offered) + (%OM X %DM3 feed refusal)] / 2] - (% OM x DM3 faeces)] x 100.

Chapter 4 The effect of a yeast-based feed additive on heat stress responses in Merino wethers

4.1 Introduction

Changes in climatic conditions can be rapid and extreme as sheep are transported from Australia to the Middle East region by sea. The sheep originate from different climates areas around Australia and are kept on the ship during voyages under ambient temperatures between 20°C and 32°C and relative humidity ranging from 70% to 90% (Black *et al.* 1994). Ambient temperatures in the low-lying coastal lands in the Middle East vary depending on the season and location. Ambient temperatures range from 40°C to 50°C during the summer and in the winter are above 30°C during the day with night not much cooler. The humidity is always above 70% (Humphreys *et al.* 2003).

During a 23-d sea transportation, sheep are offered dry lucerne (*Medicago sativa*) pellets (Black *et al.* 1994) that are a good source of crude protein and digestible energy. Feeding sheep with dry lucerne pellets is still practiced up to the present. The pelleting process increases the density of lucerne and intake is higher as the feed density increases (Pond *et al.* 2005).

High ambient temperatures reduce DMI, live weight gain and nitrogen balance but increase rectal temperature and respiration rate (Dixon *et al.* 1999; Hogan *et al.* 2004; Preston and Leng 1987). Heat increment, produced from digestion and metabolism processes, makes an additional contribution to heat load when external temperature is high (West 1999). Morand-Fehr and Dorean (2001) argued that reduction in feed intake, digestion and metabolism are thermoregulatory responses that help reduce heat production, especially that from rumen fermentation.

In a study of pre-embarkation risk factors for sheep deaths during export by sea, Norris *et al.* (1989b) concluded that failure of sheep to eat pellets in the Australian feedlot was

a risk factor for later shipboard deaths. From results from 6 voyages, inanition was reported as the major cause of death (32%). At post-mortem these sheep had less than 100 g solid matter in their reticulo-rumen (Richards *et al.* 1989). Most of the sheep that died of starvation were those which had a weight loss of around 25% of their initial live weight while in the feedlot (Bailey *et al.* 1992). There have been claims that a commercially produced yeast-based feed additive can reduce the effects of heat stress and inanition in sheep housed under hot climatic conditions.

The general objective of this study was to investigate, in both cool and hot conditions, the behavioural and physiological responses of Merino wethers to the addition of this yeast-based feed additive to a diet of lucerne pellets. The experiment was designed to assess the effect of the supplement on three factors:

- Body temperature and respiration rate
- Feed intake, feed intake pattern of dry lucerne pellet and water intake
- Excreta production, feed digestibility and live weight gain

4.2 Materials and methods

4.2.1 Housing and feeding of the experimental sheep

Sixteen Merino wethers approximately one year old, mean live weight 40.4 (± SD 4.53 kg), were housed in the 'King Ranch' climate chambers located at the University of New England, Armidale. Immediately prior to the study, the wethers were drenched with ivermectin (Ivomec; Merck Sharp and Dohme; 2.5 ml/10 kg live weight) at the University farm as part of farm's regular parasite control program. Sheep were allocated to treatments according to live weight.

The trial was conducted in a cool room and an adjacent hot room (8 sheep per room). The experiment was conducted over 20 d (4 days' adaptation plus 16 days' data collection). The adaptation period was to allow for sheep to acclimatise to the temperature and humidity and to assess their *ad libitum* feed intakes. It is regrettable since 4 days is not long enough to adapt to ruminal bacteria especially cellulolytic bacteria to the new supplement. The 16-d experiment was conducted as a 2 x 2 factorial design. There were two room temperatures (hot and cool) and two diets (lucerne pellets offered with or without the feed additive).

The rooms were insulated. Each room had individual temperature and humidity controls located within the room. Cooling, heating and humidity were controlled by an outside temperature unit. Inlet and outlet vents were adjusted to create an air-circulation system. Insulating double glass was used in the monitoring window. Lighting was by two fluorescent lamps on a timer that came on at 0700 h and went off at 2000 h. Between 2000 h and 0700 h, lights outside the rooms were kept on and provided a dull light within the climate rooms through the viewing window. Ambient temperature in the heated room was adjusted gradually from 25°C to 35°C over 20 d to allow the sheep to acclimatise after they were first placed in the room (Figure 4.1).

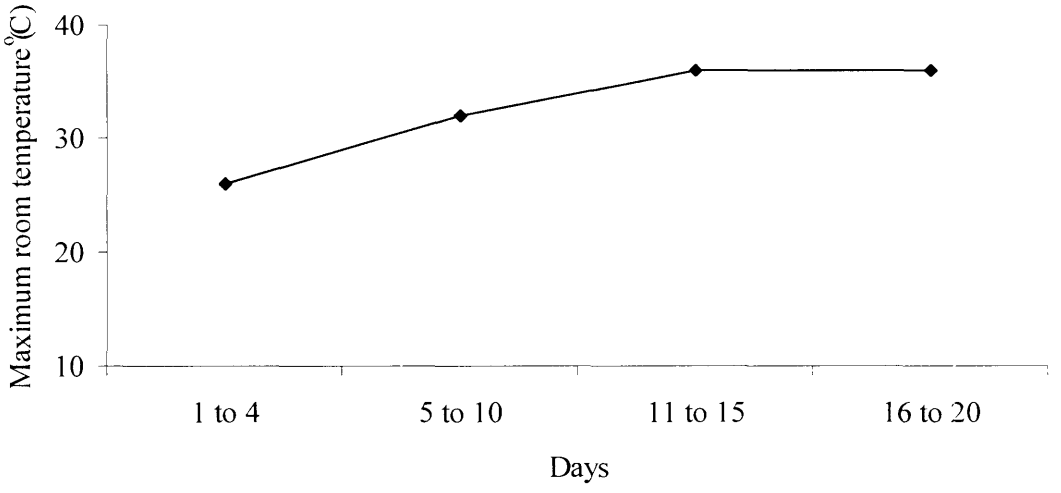

Figure 4.1 Maximum temperature in the hot room throughout the experimental period

Two dry-wet bulb thermometers, one minimum-maximum thermometer and another digital thermo-humidity meter were placed in each room. During this period, temperature and humidity were recorded three times a day at 0900, 1300 and 1700 h. The cooled room was maintained at 18°C and 60% relative humidity (RH). The heated room was maintained at 25°C between 1800 h and 0900 h. Between Day 11 and Day 20, after 0900 h the temperature was gradually increased to a maximum of 35°C and then gradually reduced to 25°C by 1700 h. As the temperature increased, RH decreased. The temperature changes in the hot room was intended to reflect conditions experienced by sheep in the post-discharge phase of the live export trade to the Middle East.

Table 4.1 Description of treatments in experiment one

Treatment	Room	n	Feed
"Cool control"	Cool	4 sheep	Lucerne pellets
"Cool + feed additive"	Cool	4 sheep	Lucerne pellets + 5 g/hd/d feed additive
"Hot control"	Hot	4 sheep	Lucerne pellets
"Hot + feed additive"	Hot	4 sheep	Lucerne pellets + 5 g/hd/d feed additive

The dry lucerne pellet used was chosen because it is the diet used in the live animal export trade. The feed additive used was a commercial product 'Farmer Peck's Yeast Supa Enzyme Supplement' (The Animal Performance Enhancing Products Pty Ltd, South Australia). It contains the active constituent alpha amylase (IUB No 3.2.11) 1000 ICNU 1 kg, inactive probiotic and Yikangsu (oligosaccharide). The yeast-based feed additive (YBFA) powder was added at the rate suggested by the manufacturer (5 g/hd per d), then well mixed with the pellets before being offered to the sheep. The nutrient components of the diets are shown in Table 3.1. The procedure used for feed intake measurement was explained in Section 3.5.

4.2.2 Measurement of body temperature and respiration rate

Body temperatures and respiration rates were assessed from Day 11 to Day 20. Temperature was recorded for 3 different parts of the animals' body, namely wool, skin, and ear, in response to the changes in the conditions. Measurement was made twice daily, just before 0900 h to represent cooler hours and then just before 1700 h to represent hotter hours. Wool-tip temperature was measured on the mid-side, head and

withers. Skin temperature was measured on mid-side and withers. Ear temperature was measured on the outer ear and inside the ear. Wool, skin and ear temperature were all measured by infra-red thermometer. When using the infra-red thermometer, the distance between the thermometer and the object was consistently 30 cm. The length of the thermometer is about 30 cm so that it was used as the standard of the desired distance.

Respiration rate was measured by observing the movement of the flank. A stop watch was used to determine the length of time taken by the sheep to inhale 30 times. This measurement was then converted to breaths per minute (bpm).

4.2.3 Measurement of feed intake, feed intake pattern and water intake

Feed-intake patterns were assessed from Day 14 to Day 20. From Day 1 to Day 4, diets were offered *ad libitum* to the experimental animals. Feed intake was measured each day and the 4-d average intake was calculated. The highest average daily intake of one sheep (2.5 kg/d) was selected as a standard amount to be offered to each animal each day during the data-collection period. During the data-collection period, the treated animals were also offered yeast-based feed additive (5 g/hd per d). The lucerne pellets were weighed ("AND" electrical balance-EP-40 KA – max 40 kg) and offered to the sheep at 0900 h each day. Total feed refusals (both the pellet and the feed additive) were weighed and recorded every morning then a sub-sample of 10% was taken, placed in a plastic bag, sealed and stored in a cool room (4°C) for later nutrient analyses and DM analyses. Feed intake was measured as the difference between the amount of feed offered and the refusals in a 24-hour period.

To determine feed-intake patterns, feed intake was recorded at 0900, 1200, 1700 and 1930 h on Days 14 to 20 and an average feed intake rate (g/h) was calculated for each period (0900 – 1200 h, 1200 – 1700 h, 1700 – 1930 h and 1930 – 0900 h).

Four litres of clean tap water were offered to each animal in the morning. Water was added to the troughs to replace the water removed by drinking when troughs were seen to be empty. In hindsight, this procedure was unlikely to have provided a measure of *ad libitum* intake. A water trough was placed in each room away from the sheep and used to measure water evaporation in a 24-hour period. The volume of water (ml) intake by the animal was calculated by subtracting the amount of both water remaining and daily evaporation from the amount offered, i.e.

Water intake (ml) = (ml) water offered – (ml water refusal + ml water evaporated).

4.2.4 Measurement of faeces and urine

After 24 h of collection, the faeces were removed and weighed ("AND" electrical balance-EP-40 KA – max 40 kg). After mixing thoroughly, 10% of the collected faeces were sampled and placed in a sealed and labelled container specific to each animal and stored at -20°C. At the end of the collection period the 6 frozen samples for each individual sheep were thawed, mixed together and then a new 10% sample was stored for later analysis.

A plastic bucket was placed under each metabolism crate to collect the urine produced by each sheep. The volume of urine collected (ml/d) was measured using a plastic measuring cylinder.

4.2.5 Live-weight change and feed conversion ratio

Live weight (W) was measured (Ruddweig, Km-1 electronic weighing system, New Zealand) at the commencement of the study, on Day 11 and at the conclusion of the study on Day 20. The change in live weight was calculated by subtracting the final weight from that recorded on Day 11. Data were recorded for individual sheep then the treatment means for daily gain and standard error mean (SEM) were calculated. Feed conversion ratio was calculated using the formula:

FCR = kg DM feed intake / kg live-weight gain.

4.2.6 Statistical analyses

The data were analysed by analysis of variance to determine the effects of temperature, feed additive and their interactions using the General Linear Model analysis (GLM), StatGraphics Plus, version 5.1-Professional Edition, Manugistics Inv., Rockville, Maryland, USA. Differences between means were considered significant when $P<0.05$.

4.3 Results

4.3.1 Body, skin and wool temperatures

Body temperatures were consistently higher ($P<0.05$) in the hot room than in the cool room but there were no differences ($P>0.05$) due to diet (Table 4.2).

Wool temperature of the sheep in the hot room at 1700 h and 0900 h was also higher (P<0.05) than that of the cool room. The yeast-based feed additive had no effect (P>0.05) on the wool temperature of sheep in either room. There was no interaction.

The skin temperature of the sheep in the hot room was higher (P<0.05) at 1700 h than that of sheep in the cool room at 1700 h, and both the outer and inner ear temperatures of the sheep in the hot room were higher (P<0.05) than of sheep in the cool room. Yeast-based feed additive has no effect (P>0.05) on skin and ear temperatures.

4.3.2. Respiration rate

There was no difference (P>0.05) in the respiration rate of the sheep in the cool and hot rooms at 0900 h. As the temperature increased in the hot room after 0900 h, respiration rate sheep increased to 234 bpm (hot control) and 229 bpm (hot + feed additive); these rates were higher (P<0.05) than those of the sheep in the cool room, i.e. 154 bpm and 142 bpm, respectively (Table 4.2 and Figure 4.2) but the addition of the yeast-based feed additive did not affect respiration rates of sheep in the hot room or the cool room.

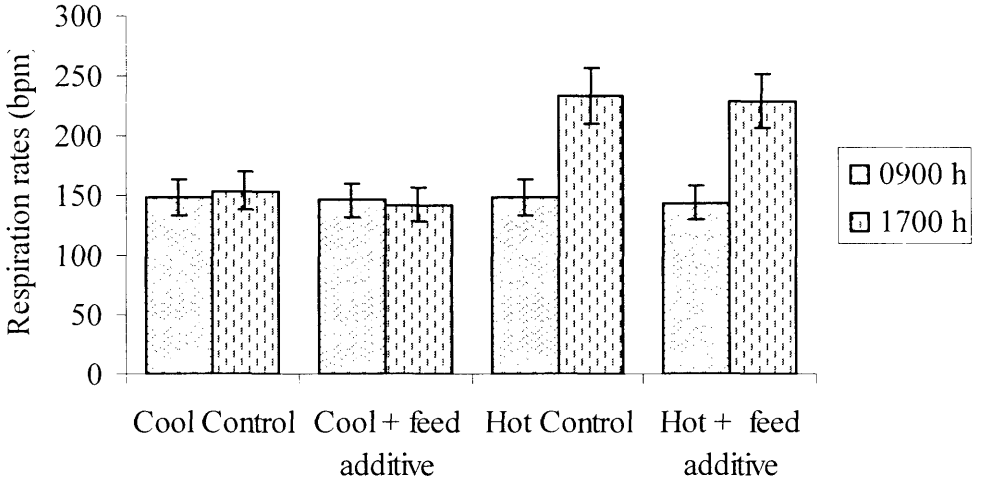

Figure 4.2 Respiration rate patterns of Merino wethers offered lucerne pellets with or without yeast-based feed additive in cool (0900 h) and hot (1700 h) climatic conditions and given lucerne hay with or without yeast-based feed additive (YBFA)

Table 4.2 Mean body temperature (°C) and respiration rates (bpm) of Merino wethers in cool and hot climatic conditions and given lucerne hay with or without yeast-based feed additive (YBFA)

Room	Cool		Hot			P values		
Treatment	Control	YBFA	Control	YBFA	SEM	Room (R)	YBFA	R * YBFA
Wool temperature (°C) from mid-side, head and wither measured at 0900 h and 1700 h								
Mid-side 0900	18.1	18.2	18.5	18.7	0.2	0.02	0.42	0.71
Mid-side 1700	16.4	16.2	35.8	35.4	0.4	0.00	0.39	0.79
Head 0900	18.1	17.7	18.4	18.4	0.2	0.04	0.41	0.54
Head 1700	16.3	16.0	35.8	35.5	0.4	0.00	0.49	0.87
Wither 0900	18.1	17.9	18.5	18.4	0.2	0.08	0.77	0.95
Wither 1700	16.2	15.9	35.8	35.5	0.3	0.00	0.47	0.91
Skin temperature (°C) at 0900 h and 1700 h								
Mid-side 0900	28.4	28.8	28.7	29.1	0.5	0.55	0.43	0.98
Mid-side 1700	27.9	28.1	37.6	37.3	0.3	0.00	0.81	0.52
Wither 0900	27.7	29.1	28.4	28.4	0.4	0.98	0.11	0.12
Wither 1700	27.2	27.9	37.5	36.8	0.3	0.00	0.91	0.59
Ear temperature (°C) at 0900 h and 1700 h								
Outer ear 0900	27.5	27.1	26.4	26.8	0.6	0.27	0.95	0.54
Outer ear 1700	25.6	25.3	37.2	37.1	0.6	0.00	0.74	0.81
Inner ear 0900	30.7	30.4	31.1	31.4	0.6	0.27	0.92	0.64
Inner ear 1700	28.9	29.4	37.8	37.2	0.4	0.00	0.88	0.25
Respiration rate (bpm) at 0900 h and 1700 h								
0900 h	149	146	148	144	3.6	0.69	0.39	0.89
1700 h	154	142	234	229	6.6	0.00	0.24	0.57

4.3.3. Feed and water intake

Water temperature at 0900 h was 16.8±0.13°C in the cool room and 17.4±0.16°C in the hot room. Water temperature at 1700 h was 17.3±0.21°C in the cool room and 30.7±0.58°C in the hot room. There was no difference (P>0.05) in DMI or water intake between the treatments when expressed as actual intakes or intakes per kgW. However, there was considerable variability in feed and water intake within treatments (SEM. 130 and 278 g/hd per d, respectively). It would have been more relevant to take several measurements over the day and take the mean.

Table 4.3 Dry matter intake (DMI; g/hd per d) and water intake (WI; ml/hd per d) of Merino wethers in cool and hot climatic conditions and given lucerne hay with or without yeast-based feed additive (YBFA)

Room	Cool		Hot			P values		
Treatment	Control	YBFA	Control	YBFA	sem	Room (R)	YBFA (Y)	R * Y
DMI	1791	1661	1519	1534	130	0.15	0.66	0.59
DMI (% of live weight)	3.9	3.6	3.4	3.3	0.3	0.16	0.52	0.84
WI	4443	4276	4453	4314	278	0.93	0.59	0.96

4.3.4. Feed intake pattern

Feed intake pattern was influenced by ambient temperature. Feed intake of sheep in the hot room was lower (P<0.05) during the hot period each day than that of sheep in the cool room but intakes of sheep did not differ (P>0.05) between rooms during the cool part of the day.

Table 4.4 Dry matter intake (g DMI/h) of Merino wethers in cool and hot rooms and given lucerne hay with or without yeast-based feed additive (YBFA)

	Cool		Hot			P values		
	Control	YBFA	Control	YBFA	sem	Room	YBFA	R * Y
0900-1200 h	190	146	128	94	246	0.04	0.13	0.83
1200-1700 h	82	67	50	54	10	0.04	0.61	0.34
1700-1930 h	64	81	102	68	14	0.41	0.56	0.09
1930-0900 h	55	59	52	50	8	0.50	0.89	0.68

Total DMI in Table 4.4 that collected from 10 days while those in Table 4.3 that collected from 16 days explains the difference of DMI between the two tables.

4.3.5. Excreta production

The total water excretion (and of its components, viz. urine, faecal water) and faecal DM of sheep in the cool room was higher ($P<0.05$) than of those in the hot room. The sheep receiving the diet with yeast-based feed additive in both rooms tended ($P=0.07$) to have higher total urine production.

Table 4.5 Urine (ml/hd per d) and faecal (g DM/hd per d) production of Merino wethers in cool and hot rooms and given lucerne hay with or without yeast-based feed additive (YBFA)

Room	Cool room		Hot room			P values		
Treatment	Control	YBFA	Control	YBFA	sem	Room	YBFA	R * Y
Urine	649	755	482	609	57	0.02	0.07	0.85
Faeces	740	722	662	598	51	0.07	0.43	0.66

4.3.6. Digestibility and production

DM and OM digestibility did not differ (P>0.05) between treatments. Average daily weight gain of sheep in the cool room was higher (P<0.05) than that of sheep in the hot room. Average daily gain of the sheep in both the hot room and the cool room was higher (P<0.05) for sheep given feed additive. Feed-conversion efficiency (FCE) of sheep in the cool room was higher (P<0.05) than that of the sheep in the hot room, but was not different (P>0.05) in sheep ingesting feed additive (Figure 4.3).

Table 4.6 Dry matter digestibility (DMD) and organic matter digestibility (OMD) and average live-weight daily gain (ADG; g/d) Merino wethers in cool and hot climatic conditions and given lucerne hay with or without yeast-based feed additive (YBFA)

Room	Cool		Hot			P values		
Treatment	Control	YBFA	Control	YBFA	SEM	Room	YBFA	R * Y
DMD	0.57	0.58	0.58	0.61	0.01	0.12	0.15	0.41
OMD	0.60	0.60	0.61	0.63	0.01	0.11	0.19	0.38
ADG	206	277	79	129	24.60	0.00	0.03	0.68

4.4 Discussion

This study showed that there was an increase of wool, skin and ear temperature when Merino wethers were exposed to hot climatic conditions. This finding agrees with that reported by Woodgate *et al.* (2001) who also found that fleece surface, skin and ear surface temperatures of sheep in 'high' ambient temperature (34.7°C) were significantly higher than those in 'low' ambient temperature (21.0°C).

The increase in respiration rate in this study indicated that the sheep in the hot environment were subjected to severe heat stress (see Table 3.3). Similar increases in response to heat stress have been reported in sheep (Dixon *et al.* 1999; Hogan *et al.* 2004) and goats (Maloiy *et al.* 1971) and in lambs, ewes, foals and mares (Piccione *et al.* 2002b).

Several studies of respiration rates of sheep have been reported which differ from the results recorded in this study. Other factors that can affect respiration rates besides the ambient temperature (Hogan *et al.* 2004) are protein supplementation (Dixon *et al.* 1999), walking activity, shorn or full fleece and wind speed (Gebremedhin *et al.* 2002; Macfarlane *et al.* 1966) and sedentary or fit sheep (Sakurada *et al.* 1998). The absence of the interaction between the effects of ambient temperature and the yeast-based feed additive in this study suggests that the inclusion of the feed additive in the diet did not affect the respiration rate of sheep and therefore probably did not reduce heat stress in these sheep.

Animals typically respond to the stress of high temperatures by reducing their DMI with a corresponding reduction in performance (NRC 1981) but increase their water intake (Hogan *et al.* 2004; Westra *et al.* 1976). By contrast, daily DMI and water intake of the Merino wethers were similar ($P>0.05$) at the high ambient temperature in this study. However, DMI was reduced in the sheep in the hot room during the hotter part of the day and the yeast-based feed additive did not improve DMI in this period. An important finding was that there is no difference in DMI and water intake between sheep offered yeast-based feed additive and their controls, whether housed under hot or cool conditions. Thus there was no evidence for the Supplier's suggestion that the additive can help to maintain higher DMI in sheep in hot conditions. However, DMI that ranges from 3.3% to 3.9% of body weight and water intake above 4 L/hd per d are

within the normal ranges for Merino sheep (Fraser *et al.* 1990). Rate of feed intake was lower ($P<0.05$) during 0900 h – 1700 h.

The lack of a difference in DMI can be explained because the negative effect of heat stress would likely become less significant when diets of high potential digestibility were offered (Beede *et al.* 1986; Bhattacharya *et al.* 1974). The lack of difference could also possibly be related to the feed intake pattern under the diurnal temperature conditions imposed. Although DMI was low during the period of high ambient temperature, the sheep ate to fulfil their DMI requirement of body weight during the cooler hours. Even though DMI was different ($P<0.05$) between room temperatures during the hottest hours, there was no difference ($P=0.41$) between 1700 h and 1930 h and ($P=0.49$) from 1930 h to 0900 h. Similarly, there was no difference ($P>0.05$) in DMI of sheep caused by yeast-based feed additive during the hottest part of the day. This finding is supported by the observations of other workers (McDowel 1972). Because there were a large differences among treatments, the daily DMI and water intakes in this study were more likely due to reflect the extent of acclimation of individual sheep to conditions in the hot room and the type of feed offered.

This study highlights the reduction in feed intake when the ambient temperature becomes a stressor. This observation is in line with the report of Leibholz (1985) who showed that sheep at 18°C had completed 88% of their feeding for a 24-hour eating period within 6 hours of feeding, while the volume was only 68% for the sheep at 35°C.

This study recorded that water intake did not increase in response to higher ambient temperature. This is more likely due to the frequencies and the amount of water offered. Water was not offered freely. Water troughs were filled up manually so that sometimes the troughs were found empty. Sheep did not drink as much as they need to satisfy their needs triggered by the higher ambient temperature.

The results also indicate that water loss was increased at higher ambient temperatures probably to meet the additional water losses through respiration (panting) and evaporation through the skin (Table 4.2). The urine output of sheep in this study are in agreement with the report of Macfarlane *et al.* (1966) who found that heat load reduced the urine output of sheep. Macfarlane *et al.* (1960) also showed there was a downward trend of urine production in sheep experiencing continuous dehydration.

There was one apparent effect of the feed additive. The total urine output tended ($P = 0.07$) to be higher in the sheep offered the yeast-based feed additive. This might be due to the two main nutrients contained in the yeast-based feed additive, namely, crude protein and water-soluble vitamins. The quantities ingested are relatively small but either of these two nutrients have had physiological effects on the sheep. The higher the concentration of crude protein in the ingested feed, the more urine is needed to eliminate ammonia and urea (Dixon *et al.* 1999). One of the water-soluble vitamins was niacin or nicotinic acid. It functions as a constituent of two important enzymes, nicotinamide adenine dinucleotide and nicotinamide adenine dinucleotide phosphate both of which have critical roles in energy metabolism. These niacin-containing enzymes are important carriers of high-potential electrons (and hydrogen) from substrates to molecular oxygen resulting in water formation (Pond *et al.* 2005). This results in the production of 'metabolic water' that is another input of water to the body water pool.

The present study indicated that differences in the ambient temperature and the yeast-based feed additive had no effect on feed digestibility. This could have been because the temperature and humidity conditions were not excessively stressful to the sheep (Church 1975). In other words, the degree of stress may not have been large enough to cause a difference in digestibility (Christopherson 1985).

Regarding feed efficiency in the hot room, the lower average daily gain of the sheep in the high ambient temperature might be explained by the use of energy for heat dissipation through respiratory evaporation rather than for increasing body mass. Sheep in this study recorded a higher respiration rates in the hot room than sheep in other studies. Another explanation for lower daily gain could be related to body water content. Although the design of this study did not permit measurement of the amount of water lost by panting, other studies confirm that body water is a major component of live weight. Merino sheep kept at 43.4°C dry bulb and 36-37°C wet bulb have been reported to gain weight, presumably due to water retention (McDonald and Macfarlane 1958). There was a decrease of live weight of as much as 11.5% exhibited by 3-d dehydrated sheep and 19.2% after 6 d of dehydration (Macfarlane *et al.* 1960).

Conclusion

In summary, the inclusion of the yeast-based feed additive at a rate of 5 g/hd per d in a lucerne-based diet provided benefits for production. In the present study, the inclusion of the feed additive in the diet increased growth rates in both hot and cool climatic conditions. The inclusion of the feed additive also appeared to increase urine output although the reasons for the increase were not clear. There is a need for future research to investigate the physiological mechanisms underlying the higher live weight gains so that the effects can be exploited.

Chapter 5 Responses of Merino wethers in hot and cool conditions to the temperature of drinking water

5.1 Introduction

The percentage of water in total body weight for ruminants ranges from 57 to 79% (Yousef *et al.* 1985), it is lower in fat animals and higher in leaner animals. Water has two basic functions in all terrestrial animals; it has major roles in body metabolism and in controlling body temperature. Water is required for digestion and metabolism of energy substrates and nutrients, transport of nutrients and metabolites to and from cells in blood, excretion of waste products, maintenance of proper ion, fluid, and heat balance, and as a fluid environment for the developing foetus (Beede 2005). These functions result from the unique properties of water (Pond *et al.* 2005). One critical property of water relating to the metabolic process is that water is a solvent (McDonald *et al.* 2002; Pond *et al.* 2005). Properties of water that allow it to have a marked effect on body temperature regulation are its high specific heat, high thermal conductivity, and high latent heat of vaporisation, which allows it to provide a means of respiratory cooling (Pond *et al.* 2005).

In natural conditions, water is heated by solar radiation or high ambient temperature. When ambient temperatures are above 30°C, water temperature in a shaded trough was about 7°C lower than the unshaded water. Drinking water with a temperature difference reduced the heat load on lambs (Holst and Stanley 2000). It might be expected that heat-stressed sheep would not be likely to drink hot water when they are trying to reduce their heat load but would prefer to drink cool water; however, experimental evidence suggests this does not appear to be the case (see below).

Only a small number of studies have been conducted to measure the effect of drinking water temperature on animal production. Animals studied have included lactating goats (Olsson *et al.* 1996), Hampshire wethers (Brod *et al.* 1982), non-lactating Holstein cows

(Cunningham *et al.* 1964), and sheep (Bailey *et al.* 1962; Brod and Bolsen 1979). Unexpectedly, heat-stressed lactating goats preferred to drink warm (35°C) rather than cooler (15°C) water (Olsson *et al.* 1996). Rumen temperature depression of non-lactating Holstein cows depended upon the amount and temperature of ingested water with the greatest depression being in response to water at 0°C water (Cunningham *et al.* 1964). Water temperature had no significant effect on nitrogen balance, or digestibility of crude fibre and crude protein, although the lowest coefficients were in the 0°C treatment group. Volatile fatty acids and ammonia-N concentrations in the rumen were depressed by the 0°C water treatment (Brod *et al.* 1979; Brod *et al.* 1982). As ambient temperature fell from 15°C to -12°C, the rumen, rectal and subcutaneous tissue temperatures decreased. Water intake also decreased from 1600 to about 800 ml/d. The temperature of the drinking water did not affect water intake at ambient temperature of -12°C. When the drinking water was 0°C, rectal temperature was higher than when it was 30°C (Bailey *et al.* 1962) which seems counter-intuitive.

These studies reveal that, as the animal drinks different volumes of water of different temperatures, changes occurred in rumen, rectal and subcutaneous temperature and physiological functions that in turn dictate feeding and drinking responses and other behaviours. The effects of drinking water temperature on Merino wethers that are subjected to higher ambient temperature have not been studied.

The aim of this study was to test the hypothesis that for sheep an increase in temperature of drinking water in a hot room would result in a decrease in their water intake but an increase in water intake in the cool room. It was hypothesised that these responses would assist the maintenance of a normal body temperature, promote normal functioning of rumen fermentation and increase feed digestibility. Accordingly, Expt 2 experiment was conducted to determine the effects of ambient conditions (temperature and relative humidity) and different drinking water temperature on body temperature, respiration rate and urine output, and on rumen fermentation and digestion in Merino wethers. Diurnal patterns of feed intake and drinking water were also recorded.

5.2 Materials and methods

Eight sheep (2 years of age) were selected from the University farm 'Trevenna', and treated for internal parasites and then were moved to pens in the physiology building for surgery. The room temperature in the surgery pens was a maximum of 26°C and a

minimum of 23°C. Sheep were offered lucerne chaff *ad libitum* and given continuous access to water.

The climate laboratory rooms

During the experiment, the sheep were housed in climate rooms in the animal house of the University of New England, designated 'cool' and 'hot' rooms. Each room was 4 m x 4 m in size and heated with hot water pumped from heated tanks outside the rooms. A computer-controlled thermostat opened and closed valves controlling the amount of water, either cool or hot, to be pumped into water jackets in the walls of the climate-controlled rooms. The walls of the rooms were covered by metal.

The rooms were illuminated during the day from 0600 h to 2000 h but were dark over night. Room temperature and relative humidity were recorded automatically using data-logging software on a computer running continuously. In addition, a wet and dry bulb thermometer was placed in each room for manual recording.

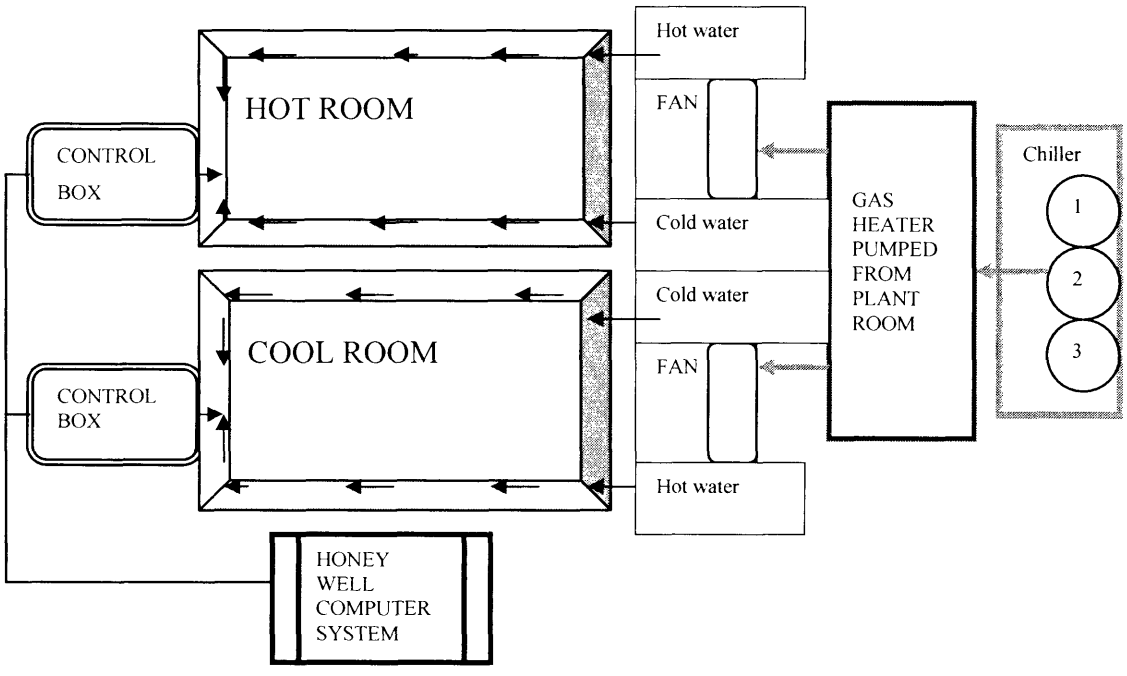

Figure 5.1 Diagram of the climate room layout

Room temperature in the cool room was set at 20°C and remained stable during the adaptation (16 - 25 October 2006) and data collection periods. In the hot room, temperature was adjusted gradually from 20 to 40°C during the day through adaptation period. The adjustment was changed during data collection where ambient temperature

was set to 40°C during the day (0600 h to 1800 h) then was reduced to 30°C at night. Daily pattern of room temperature and relative humidity during the data collection is presented in Figure 5.2. The 24-h ambient temperature in the cool room was relatively stable. Relative humidity was also stable, except for a slight increase during the night. In the hot room, there was an intersection between room temperature and relative humidity where relative humidity increased at night as the ambient temperature fell.

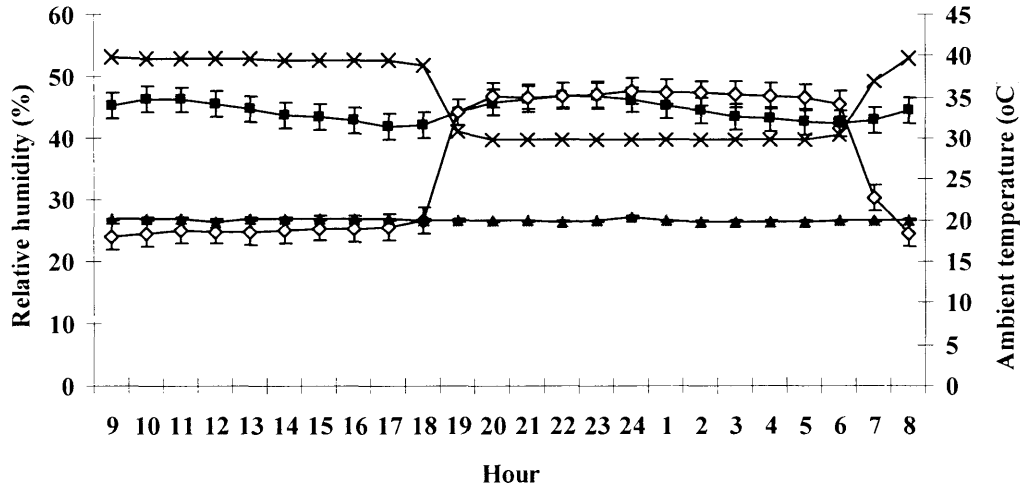

Figure 5.2 Mean daily room temperature (°C) and relative humidity (%) of climate rooms during the days of data collection. ■----■ RH cool room, ◊----◊ RH hot room, ▲----▲ ambient temperature cool room and ×----× ambient temperature hot room

5.2.1 Animals and treatments

The 8 sheep were blocked according to live weight and randomly divided within blocks to two groups (hot room, n=4; cold room, n=4) of treatment. The average live weight of the 8 sheep was 47.0 kg (SD 3.5 kg) and the length of fleece was 2.5 - 3 cm. Within each room, four metabolism crates were equipped to offer drinking water at different temperatures as follows: 20°C (crate 1), 30°C (crate 2), 40°C (crate 3) and a choice between two drinking troughs, i.e. 20°C and 30°C (crate 4).

All sheep were offered lucerne (*Medicago sativa*) chaff *ad libitum* and had continuous access to water (Section 3.5).

The 36-d study was divided into an adaptation period (4 d) and measurement period (32 d). The measurement period comprised of 4 sub-periods of 8 d (1 d adaptation and 7 d measurement). Adaptation period is relatively short because physiological changes especially packed cell volume was need to be measured every day during the sheep were subjected to hot climatic conditions. The 4 sheep were rotated between the metabolism crates within each room at the end of each measurement period so that each sheep was subject to all drinking water treatments according to a Latin Square design.

Each metabolism crate was equipped with an individual 20-litre plastic container from which drinking water was circulated at 1200 L/h continuously into its water trough using a pump (Kongs International Co., Ltd, Australia). The water at 20°C in the cool room was dependent upon the ambient temperature and that at 30°C in the cool room by a 100 Watt aquarium tank heater (Digi-therm heater, Pet Pacific PTY Ltd-Sydney, Australia). Water at 40°C in both rooms was pumped to and from a water bath. Drinking water temperatures of 20°C and 30°C in the hot room were also maintained using Digi-therm heaters.

A standard glass thermometer was placed in all water troughs so that the water temperature could be recorded any time. Water evaporation was measured 3 d after the animal was released from the crates at the end of experimentation. Later, the evaporation was used to correct the amount of water consumption.

5.2.2 Measurement of body temperature and respiration rate

Rectal temperature was used as the measure of body temperature. Rectal probes were inserted 7 cm into the rectum of each sheep. Rectal probes were connected to data-loggers (Power labs) that recorded rectal temperature onto the computer in 1-second intervals on a 24-h basis. Gut and carotid temperatures were not recorded for this study.

Internal body temperature was measured using calibrated thermistors connected to a computer (PC XP Chart v5.4.2 ADInstruments Pty Ltd Unit 13, 22 Lexington Drive Bella Vista NSW 2153 Australia) in each room.

Outer body temperatures, i.e. wool, skin, nose and ear temperature, were measured using an infra-red digital thermometer. However, data of these recordings are not presented as a part of this Expt. 2 since the core body temperature is received much more attention.

Respiration rate in the hot room was measured by a pressure transducer (C. Palmer, England) attached to a girth strap placed around the sheep at chest level. In the cool room the rate was measured manually by counting the movements of the flank, using the same procedure as described in Section 4.2.

5.2.3 Measurement of feed and water intake

The feed was weighed (digital balance, mark "Bonso" model- 322, capacity 2000 g, graduation: 1 g) four times a day. The daily DM feed intake was calculated from the amount offered minus the amount refused for 24 h (Section 3.5).

Water consumption patterns from crates 1, 2 and 4 in each room was measured 4 times a day by weighing water in the plastic container using an electronic balance (Sartorius, type 1507). That in crate 3 was measured using an electronic balance ("AND" electrical balance-EP-40 KA – max 40 kg). Total daily consumption of water was calculated as the difference between water offered and water refused, corrected for daily evaporation.

5.2.4 Measurement of faeces and urine production

In this study, faeces were separated from urine using a separator under each metabolism crate. After 24-h total collection, faeces were well mixed, weighed (Sartorius, type 1507) and 10% aliquots were pooled, oven dried at 60°C until the weight was constant (48 h). Seven-day samples from each sheep for every period were bulked and a representative sample was taken and ground to pass 1 mm sieve for further analysis (Section 3.8).

The volume of urine produced over the 24-h period was collected into 100 ml 10% (v/v) H_2SO_4 as described by Chen and Gomes (1992). However, if the pH measured was higher than 3, the quantity of acid was increased to either 200 or 400 ml. Samples of the 24-h collection were mixed thoroughly and 2% of each day's urine was added to a plastic container to give a 7-d bulked sample that was stored at -20°C pending later analysis (Section 5.2.8).

For pH determination, urine samples were collected in the morning without acid, pH urine was determined (Eutech Instruments Ecoscan) and then the remaining urine was transferred into the bucket with acid for the remainder of the 24-h collection.

5.2.5 Measurement of live-weight change

Live weight was measured (Ruddweig, KM – electronic weighing system, New Zealand) at the start and end of each period. The difference between final live weight and initial live weight divided by day units gave the average daily live weight gain.

5.2.6 Blood collection and measurement of packed cell volume (PCV)

Blood samples (10 ml) were collected every day approximately 3 h after the morning feeding for 7 d in each period. Samples were collected by jugular venipuncture into heparinised plastic tubes (Lithium heparin 125 I.U) and immediately processed to obtain packed cell volume (PCV). The PCV was determined by the micro-capillary method (Guerrini *et al.* 1982). Blood samples were drawn into micro-capillary tubes (75 mm/75µl d.e 1.5 – 1.6 mm, Germany code 7301) which were centrifuged (Heraeus Sepatech) at 14000 rpm for 10 min. A reader (Hawksley Micro-hematocrit reader, England) was used to read the PCV value. Each blood sample was analysed in duplicate.

5.2.7 Analytical procedure and calculations

Urine nitrogen

The frozen urine samples were thawed and mixed thoroughly to provide a representative sample for nitrogen determination. Urine total nitrogen was determined using a LECO® FP-2000 automatic nitrogen analyser (Leco Corp., St Joseph MI, USA). The nitrogen values were converted to the crude protein equivalent by multiplying by the factor 6.25.

Nitrogen balance

Nitrogen balance was determined by subtracting the combined faecal and urine N losses from the total N intake. Nitrogen concentration in faeces, urine and feed was determined using a LECO® FP-2000 automatic nitrogen analyser.

Allantoin excretion

Allantoin excretion (mg/d), predicted microbial outflow from the rumen (g/d) and efficiency of microbial synthesis (g/DOMI) were determined using the procedure proposed by Chen and Gomez (1992).

5.2.8 Statistical analysis

Data were analysed according to a 4 x 4 Latin square design, i.e. the four periods in which each sheep in turn received 1 of 4 water temperature treatments. The main effects of ambient temperature and drinking water temperature and their interactions were determined using Statgraphics version 5.1. The General Linear Model (GLM) was used with Analyses of Variance. Means were separated using 95% Least Significance Difference (LSD) confidence intervals. The P values less than or equal to 0.05 were considered significant.

5.3 Results

Measurement of dry bulb temperature in each room showed that ambient temperatures were ± 1°C of the set temperature (20°C for the cool room and 40°C during the day and 30°C during the evening for the hot room). Water temperature records taken 4 times daily showed that water temperatures were also within ± 1°C of the desired water temperature for each treatment.

Mean respiration rate of sheep in the hot room at 1500 h (mean = 206 bpm) was higher ($P<0.05$) than for sheep in the cool room at 1500 h (mean = 149 bpm). Mean 24-h rectal temperature of sheep across the experimental period in the hot room (40.7°C) was higher ($P<0.05$) than for sheep in the cool room (40.0°C).

Mean daily DMI of sheep was higher ($P<0.05$) in the cool room (1578 g) than in the hot room (1136 g). DM intake expressed as a percentage of sheep live weight (W) was higher ($P<0.05$) for sheep in the cool room (3.0%) than in the hot room (2.4%). OM digestibility was higher ($P<0.05$) for sheep in the cool room (64%) than for sheep in the hot room (62%). There was no difference in live-weight change of sheep between the hot room and the cool room (Table 5.1).

Table 5.1 Comparison of mean live-weight change, daily DMI (DMI), daily organic matter intake (OMI), dry matter digestibility (DMD) and organic matter digestibility (OMD) for sheep housed in a cool room (20°C) or a hot room (40°C day time; 30°C night time).

	Livewt change (kg)	DMI (g/d)	DMI (% livewt)	OMI (g/d)	DMD (%)	OMD (%)
Cool room	-1.1	1578 [a]	3.0 [a]	1436 [a]	62	64 [a]
Hot room	-0.5	1136 [b]	2.4 [b]	1034 [b]	60	62 [b]

[ab] Means within columns are different (P<0.05)

Total daily water intake of sheep was higher (P<0.05) in the hot room (8.28 L) than in the cool room (5.83 L) (Table 5.2). The ratio of total daily water intake to feed intake (DM basis) was higher (P<0.05) for sheep in the hot room (7.7) than for sheep in the cool room (3.9). Total daily faecal DM output of sheep was higher (P<0.05) in the cool room (598 g) than in the hot room (443 g) (Table 5.2). Total daily faecal water excretion of sheep in the cool room was higher (P<0.05) (0.98 L/d) than in the hot room (0.71 L/d). Total daily urine production of sheep in the hot room was higher (P<0.05) (4.59 L) than in the cool room (2.25 L) (Table 5.2). Urine pH (measured daily) was higher (P<0.05) for sheep in the hot room (9.1) than for sheep in the cool room (8.7).

Blood packed cell volume (PCV) of sheep was not affected by room temperature, water temperature and there was no significant interaction. When measured on the last day of each of the four periods, rumen pH of sheep was higher (P<0.05) in the cool room (6.4) than in the hot room (6.1) (Table 5.2). Mean rumen pH for all sheep reduced (P<0.05) in the period after feeding from 6.5 at feeding time to 6.1 by 13 h post-feeding.

Table 5.2 Comparison of mean daily water intake (WI), ratio of water intake to dry matter intake (DMI), faecal production, urine output, urine pH, blood packed cell volume (PCV) and rumen pH for sheep housed in a cool room (20°C) or a hot room (40°C day time; 30°C night time).

	WI (L/d)	WI : DMI	Faeces (g DM/d)	Urine (g/d)	Urine pH	Blood PCV	Rumen pH
Cool room	5.83 [a]	3.9 [a]	598 [a]	2.25 [a]	8.7 [a]	29	6.4 [a]
Hot room	8.28 [b]	7.7 [b]	443 [b]	4.59 [b]	9.1 [b]	28	6.1 [b]

[ab] Means within columns are different (P<0.05)

Total daily nitrogen intake of sheep was higher (P<0.05) in the cool room (50.7 g) than in the hot room (36.2 g) (Table 5.3). Faecal nitrogen (N) concentration and output of sheep was higher (P<0.05) in the cool room (2.06%, 12.4 g N/d) than in the hot room (2.01%, 8.9 g N/d). Urinary nitrogen concentration of sheep was higher (P<0.05) in the cool room (1.4 % N) than in the hot room (0.6 % N). Total daily nitrogen excretion of sheep was higher (P<0.05) in the cool room (42 g) than for sheep in the hot room (32 g) (Table 5.3). Total nitrogen digestibility was not affected by room temperature, water temperature or their interaction. The nitrogen balance of sheep was higher (P<0.05) in the cool room (8.2) than in the hot room (4.4) (Table 5.3).

Table 5.3 Comparison of mean daily nitrogen (N) intake, faecal N concentration, urinary N concentration, total N excretion, N digestibility (ND) and N balance (NB) for sheep housed in a cool room (20°C) or a hot room (40°C day time; 30°C night time).

	N intake (g N/d)	Faecal N (g N/d)	Urinary N (g N/d)	N excreted (g N/d)	ND (%)	NB (g N/d)
Cool room	51 [a]	12.5 [a]	29.9 [a]	42.4 [a]	83.1 [a]	8.2 [a]
Hot room	36 [b]	9.1 [b]	22.6 [b]	31.7 [b]	88.1 [b]	4.4 [b]

[ab] Means within columns are different (P<0.05)

Daily DMI expressed as a percentage of live weight was affected (P<0.05) by room temperature (Figure 5.3) and there was no interaction found between room temperature and water temperature. Similarly, faecal production, faecal water content, DM digestibility and OM digestibility were influenced by room temperature (P<0.05) not by water temperature (P>0.05).

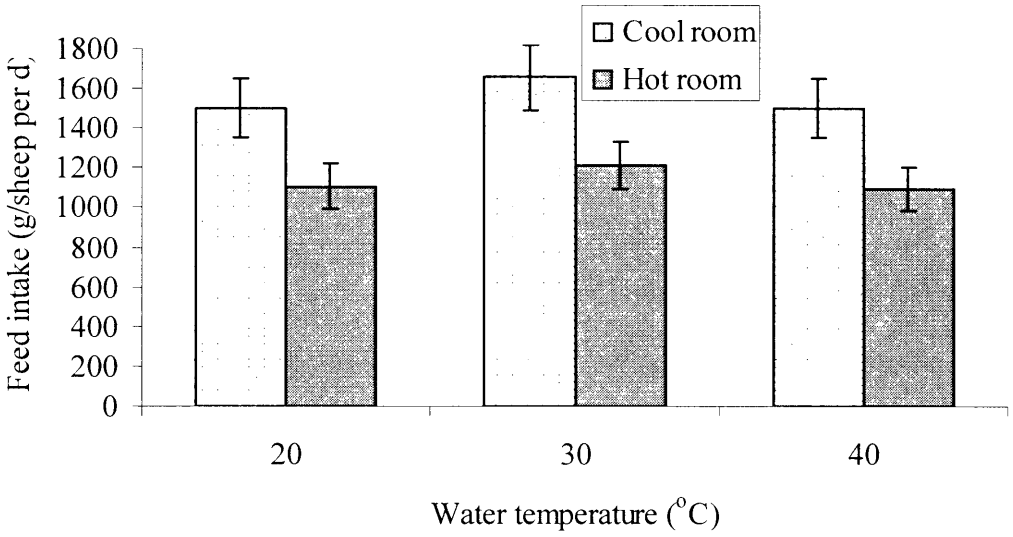

Figure 5.3 Mean daily feed intake (DM basis) of sheep housed in a hot room (ambient temperature up to 40°C) or cool room (ambient temperature 20°C) and offered drinking water at 20°C, 30°C or 40°C.

In the cool room, water temperature did not (P>0.05) affect water intake (Figure 5.4). However, in the hot room, total daily water intake increased as water temperature increased and the water intake was higher (P<0.05) at water temperature of 40°C (Figure 5.4).

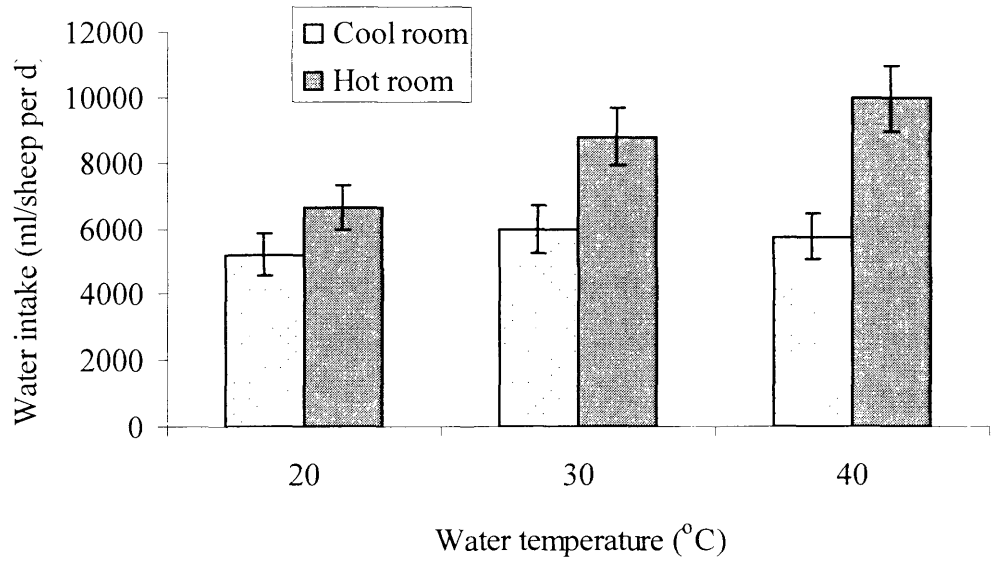

Figure 5.4 Mean daily water intake of sheep housed in a hot room (ambient temperature up to 40°C) or cool room (ambient temperature 20°C) and offered drinking water at 20°C, 30°C or 40°C.

The relationship between drinking water temperature and daily urine production of sheep in the hot room and cool room followed the same trend as that observed for water intake (Figure 5.5).

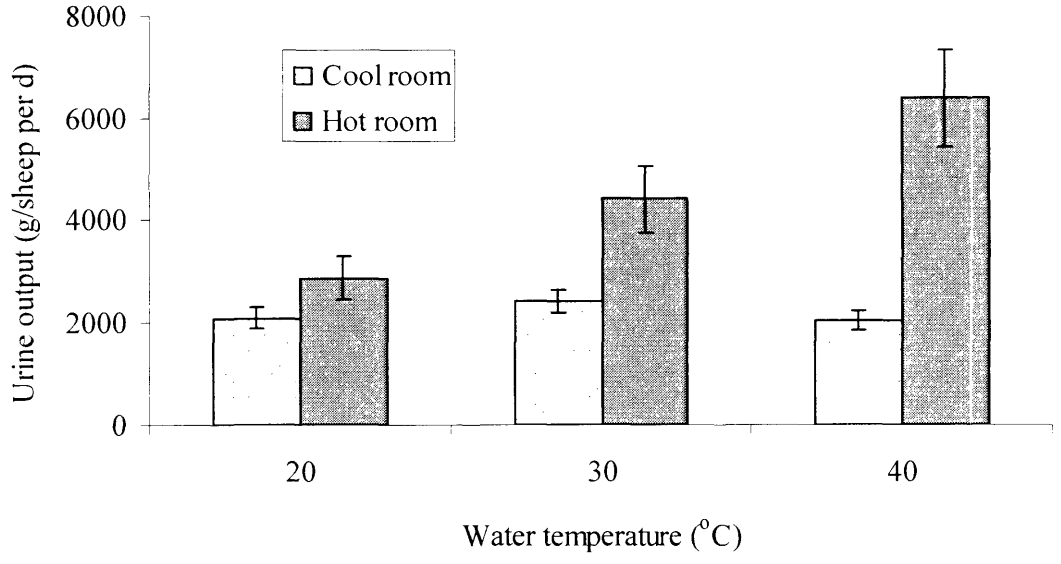

Figure 5.5 Mean daily urine output of sheep housed in a hot room (ambient temperature up to 40°C) or cool room (ambient temperature 20°C) offered drinking water at 20°C, 30°C or 40°C.

Urine pH was not affected by water temperature. There were no differences in faecal nitrogen concentration due to water temperature. Urinary nitrogen concentration, blood packed cell volume (PCV) and rumen pH were not affected ($P>0.05$) by water temperature.

Total daily nitrogen intake of sheep was not affected by water temperature. Faecal nitrogen (N) and urinary N concentration and total daily nitrogen excretion of sheep was not affected by water temperature. The nitrogen balance of sheep was not affected by water temperature.

When measured across four periods daily (0900 h to 1200 h, 1200 h to 1500 h, 1500 h to 1800 h and 1800 h to 0900 h), DMI was higher ($P<0.05$) in the cool room in all periods of the day. Sheep ingested a higher ($P<0.05$) proportion of their daily feed intake at night-time (1800 h to 0900 h) than during the day time (0900 h to 1800 h) in both the hot room and cool room. Sheep in the hot room ingested a higher proportion of their daily feed intake during the evening as water temperature increased.

Table 5.4 Percentage of daily feed intake of sheep housed in a hot room (ambient temperature up to 40°C) or cool room (ambient temperature 20°C) offered drinking water at 20°C, 30°C or 40°C occurring during day-time and night-time.

Water	Day-time (0900 h – 1800 h)		Night-time (1800 h – 0900 h)	
temp.	Cool	Hot	Cool	Hot
20°C	42	44	58	56
30°C	43	40	57	60
40°C	42	36	58	64

Means within rows during day-time and night-time did not differ ($P>0.05$) but sheep in both rooms ingested a higher ($P<0.05$) of their daily intake during night-time.

When measured across four periods daily (0900 h to 1200 h, 1200 h to 1500 h, 1500 h to 1800 h and 1800 h to 0900 h), a significant difference ($P<0.05$) of feed intake and water intake due to room temperature, water temperature and their interaction were only found during the hours of 1800 h to 0900 h. No differences were found in water intake

of sheep due to room temperature, water temperature or their interaction for the other three periods of the day. However, when these periods were analysed collectively (as 0900 h to 1800 h), room temperature, water temperature and their interaction were found to influence ($P<0.05$) water intake as a proportion (%) of total daily intake (Table 5.5).

It is apparent from Table 5.5 that more water was drunk by sheep during the night-time (1800 h to 0900 h) than during the day-time at both room temperatures and all three water temperatures. Table 5.5 also shows a pattern in which sheep that are drinking water at 40°C take in a higher proportion at night than sheep drinking water at 20°C or 30°C.

Table 5.5 Percentage of daily water intake (%) of sheep housed in a hot room (ambient temperature up to 40°C) or cool room (ambient temperature 20°C) offered drinking water at 20°C, 30°C or 40°C occurring during day-time and night-time.

Water	Day-time (0900 h – 1800 h)		Night-time (1800 h – 0900 h)	
temp.	Cool	Hot	Cool	Hot
20°C	46	49	54	51
30°C	47	48	53	52
40°C	41	44	59	56

Means within rows during day-time and night-time did not differ ($P>0.05$) but sheep in both rooms drunk a higher ($P<0.05$) of their daily water intake during night-time.

Analyses of the water preference data revealed that for sheep in the hot room, water intake was higher ($P<0.05$) from 30°C water (6708 g/d) than from 20°C water (1185 g/d). For sheep in the cool room, water intake was higher ($P<0.05$) from the 20°C water (4024 g/d) than from the 30°C water (2646 g/d).

For sheep in the cool room, water intake from both 20°C and 30°C water was highest during the evening period (1800 h to 0900 h) (Figure 5.6). For sheep in the hot room, water intake from 30°C water was higher ($P<0.05$) during the evening period and the morning period (0900 h to 1200 h) than at other times (Figure 5.6). Water intake was

lowest (P<0.05) in the cool room for both water temperatures and in the hot room at 20°C between 0900 h and 1800 h (Figure 5.6).

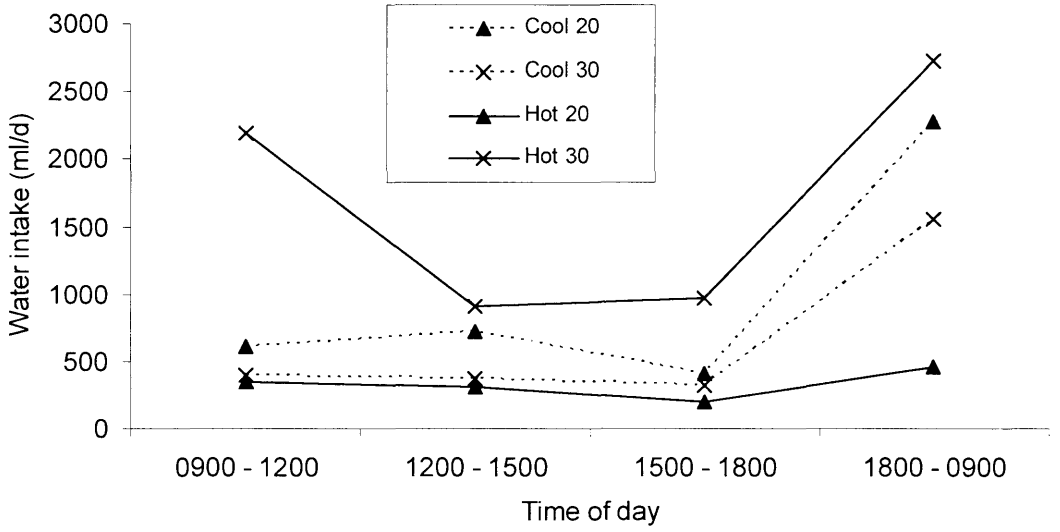

Figure 5.6 Water intake of sheep housed in a hot room (ambient temperature up to 40°C) or cool room (ambient temperature 20°C) and offered a choice of drinking water at 20°C or 30°C measured over four periods each day.

Allantoin excretion and therefore the predicted microbial outflow from the rumen was higher (P<0.05) in the cool room (22 g/d) than in the hot room (16 g/d). In contrast, efficiency of microbial synthesis (g/g DOMI) was not affected (P>0.05) by room temperature (25 g microbial N/ kg DOMI).

Table 5.6 Microbial N (g/d), efficiency of microbial synthesis (gN/kg DOMI) and and allantoin excretion (mmol/d) of sheep in a hot room or cool room offered drinking water 20°C, 30°C, 40°C and a choice between 20°C and 30°C

Room	Treatment				P Values		
	20	30	40	20/30	R*	T*	P*
Microbial N							
Cool	20.77	22.11	21.31	23.58	0.00	0.15	0.00
Hot	14.71	15.68	16.96	15.41			
Cool	20.77	22.11	21.31	-	0.00	0.19	0.00
Hot	14.71	15.68	16.96	-			
Efficiency of microbial synthesis							
Cool	23.49	24.43	25.54	24.55	0.33	0.07	0.00
Hot	23.65	25.35	29.18	24.04			
Cool	23.49	24.43	25.54	-	0.22	0.05	0.02
Hot	23.65	25.35	29.18	-			
Allantoin excretion							
Cool	15.08	16.00	15.42	17.10	0.00	0.17	0.00
Hot	10.57	11.25	12.20	11.08			
Cool	15.08	16.00	15.42	-	0.00	0.22	0.00
Hot	10.57	11.25	12.20				

*R = room, *T = treatment, *P = period

Allantoin excretion, the predicted rumen microbial outflow and the efficiency of microbial synthesis (gN/ kg DOMI) tended (P=0.07) to differ with drinking water temperature but it appeared that the effect of the treatment giving drinking water choice

had the large effect on the analysis. When the analysis was re-done with the 20/30°C treatment excluded, the difference were significant ($P<0.05$).

There was a difference ($P<0.05$) in total microbial outflow and the efficiency of microbial synthesis from the rumen between periods.

5.4 Discussion

The mean respiration rate of sheep in the hot room (24-h mean of 206 bpm) from the current study is indicative of severe heat stress (Silanikove 2000), especially during the day time. The mean respiration rate of sheep in the cool room from the current study (mean 149 bpm) was higher than previously recorded for sheep in comparable ambient temperatures (Srikandakumar *et al.* 2003). In the current experiment, the sheep were housed in a climate-controlled room whereas sheep used by Srikandakumar *et al.* (2003) were housed in an open pen under shade. Climatic factors such as wind velocity or lower RH (increasing water evaporation rates) and may have contributed to lower respiration rates. Sheep used in the latter study may also have been better acclimatised to the conditions.

The mean difference in mean rectal temperatures of sheep in the current study (0.7°C) indicates that sheep in the hot room were more heat stressed than those in the cool room. A rise of less than 1°C in rectal temperature has been found to reduce performance in most livestock species (Silanikove 2000). The reduction of feed intake of sheep in the hot room is further evidence that these sheep were more heat stressed than those in the cool room. Reduction in daily feed intake measured in the current study (3% vs 2.4% of live weight) was similar to that measured in related research with both sheep and cattle.

Sheep in the cool room had higher feed digestibility than that in the hot room (62 vs. 60) but the difference was not significant. Compare with results of Expt. 1 Chapter 4, DMD was also similar between cool and hot room. This finding conflicts with the findings of some previous studies (Christopherson 1985; Christopherson *et al.* 1983; Leibholz and Hartmann 1972). Higher digestibility in heat stressed animals is thought to be a consequence of lower feed intake, shorter rumination time, and longer retention time of feed in the rumen (Leibholz *et al.* 1972). In the current study, increased water intake of sheep in the hot room may have increased outflow rate of rumen fluid

(Silanikove 1992), which may have decreased the time available for microbial digestion with a resultant poorer digestibility.

Increased daily water intake in response to increased ambient temperature, as measured in the current study, is a normal response that has been reported from previous studies (Keskin *et al.* 2005). The associated increase in daily urine output was also expected as it is a consequence of the increased water intake.

The lower rumen fluid pH of sheep in the hot room compared to those in the cool room in the present study is a finding in agreement with others (Denek *et al.* 2006). The lower pH in the heat stressed sheep may have been due to their reduced feed intake, causing rapid fermentation and production of VFA, depressing rumen pH. However, other factors such as increased mineral absorption from the rumen (Emanuele and Staples 1994), saliva flow (Russel *et al.* 2001) or water intake could be reasonable explanations. The increased fermentation of feed due to the decrease feed intake likely because of the longer time of residence of digesta in the rumen.

It is reasonable to assume that nitrogen measures (intake, faecal and urinary concentration, excretion, digestibility and balance) were directly related to feed intake and digestibility. In accord with their higher feed intake, sheep in the cool room had higher nitrogen parameters than those in the hot room. While nitrogen intake and digestibility of sheep were higher in the cool room than the hot room, differences in live weight change were not detected, probably because of the limited number of measurements (4 per sheep) and large variation between animals and within treatments.

The microbial synthesis efficiencies (g microbial N/ kg DOMI) predicted from allantoin excretion in urine were similar to estimates made by other workers in sheep given diets of chopped lucerne hay (15.0, calculated from Pinares-Patino *et al.* 2003, Table 2). The lower excretion of allantoin in sheep housed in the hot conditions was probably a consequence of their lower DMI and DOMI. There were also differences in urinary allantoin, microbial outflow and microbial synthesis efficiency between the periods of the experiment. This was probably a response to changes in the nutrient composition of the lucerne chaff offered to the sheep. Feed was supplied in several batches with different nutrient contents (Table 3.2) so the feed composition differed between periods.

Drinking water temperature and the interaction between drinking water temperature and room temperature did not have significant effects on most of the measurements. The

lack of response in rectal temperature due to drinking water temperature is consistent with the results of similar studies with cattle that demonstrated a short-term effect on rumen temperature but no effect on rectal temperature (Brod *et al.* 1982). The exception to this situation applies when water temperature was below 0°C (ice) and rectal temperatures were reduced for up to 8 h (Mendel and Raghavan 1966). A more extreme range of drinking water temperatures may have resulted in significant effects on the rectal temperature of the sheep in the current study.

The water intake pattern of sheep with peak feed intakes occurring during the evening is consistent with the observations of Keskin *et al.* (2005). The strong association between feeding drinking patterns found in this study is similar to the findings of Murphy (1992). Feeding patterns in hot conditions have been shown to be influenced by heat production associated with metabolism of food (Thwaites 1967). It is therefore not surprising that sheep in the hot room tended to consume a larger proportion of their daily feed intake during the evening hours when room temperature was lower.

Sheep demonstrated a preference for cooler water (20°C) in the cool room and warmer water (30°C) in the hot room. This preference is similar to that observed in goats which preferred to drink warmer water when given a choice between water at 35^0 and 15^0C at both normal and at 40^0C ambient temperatures (Olsson *et al.* 1996). Whether cattle also prefer hot drinking water in a hot climate is unclear, with some workers finding a preference for water at 10^0C over that at 27^0C (Wilks *et al.* 1990) while other researchers found a preference for water at 28^0C over that at 10^0C (Milam *et al.* 1986). However, hotter drinking water at near body temperature was not offered in either study.

The heat loss mechanism of sheep is more similar to goats (more respiratory evaporation) than cattle (more cutaneous evaporation) and this is a likely reason for the similarity in water temperature preference observed in the sheep in current study with the preferences of goats.

A preference for warm drinking water in sheep and goats, but not cattle, could be explained as follows. First, the preference might be related to the interaction between cerebral thermal sensors and the regulatory thirst factor. Initiation of drinking apparently coincides with an increase of brain temperature (Jessen *et al.* 1998). Secondly, neurally mediated interactions between the senses (taste and smell) and post-

ingestive feedback cause changes preferences (affective value), and changes in preference cause changes in food selection (Provenza 1995). As the senses affect feed selection, the same principle may explain the preference shown by sheep to drink water at a temperature close to the sheep's body temperature so that no cold load is imposed (Jessen *et al.* 1998). Third, there are detectors of the variables regulating temperature sensitive neurons and so Olsson and his co-workers argued that perhaps goats preferred warm drinking water because of the absence of inhibitory signals from the oro-pharyngeal tract (Olsson *et al.* 1996). Lastly, the system regulating body temperature has physiological and behavioural aspects. The reglutatory system reacts sensitively to core and superficial body temperature. The temperature-sensitive neurons at the periphery (and especially near the mouth) exhibit a change in frequency of firing when in contact with water. These neurons are the mechanism that the animal uses to sense and interact with environmental factors such as warm water. Drinking warm water does not involve a complex recognition and decision mechanism, it just simply depends on these neurons (Stewart 1991).

This study has rejected the hypothesis that sheep would prefer not to drink water of 40^0C in hot climatic conditions. There was a preference in the hot room for the sheep to drink more water as its temperature increased. From this study, it seems that sheep prefer to drink water at temperatures close to ambient temperature in the hot climatic conditions typical of the summer months in the Middle East region. This is a new finding that establishes the need for further studies to determine whether the preference of sheep for hot drinking water in hot climates has a positive or negative net impact on the heat load of the animal and on its well-being and production.

Chapter 6 General Discussion

Over 4 million sheep are exported from Australia to the Middle East region annually and the period of peak demand coincides with winter months in Australia and summer months in the Middle East (Norris 2005). The rapid change (12 to 30 d) in climate during the sea voyage, departing Australia at mean ambient temperature of 14°C and arriving in the Middle East at mean ambient temperature of 43°C creates a situation where heat stress of sheep is likely. The use of feed additives (including the one tested in this study) and the provision of chilled (as low as 15°C) drinking water have been considered for implementation in the post-discharge phase of the live export trade of sheep.

The two experiments comprising the current study demonstrate that the use of a yeast-based feed additive and manipulation of drinking water temperature are potentially beneficial strategies for heat stress management of Merino sheep. A new and unexpected finding was that heat-stressed sheep chose to drink hot rather than cold water. These findings will have important application for the live export trade of Australian sheep to the Middle East region.

Ambient temperature differences in the hot room and cool room of both experiments created differences in DM intake, respiration rate and rectal temperature that are consistent with those expected in sheep experiencing severe heat stress when compared with those that are not.

Because daily feed intake and digestibility were not affected by inclusion of the yeast-based feed additive in diets of the sheep, their increased growth rate compared with their controls suggests that their energy was used more efficiently by their tissues. Heat loss of sheep (primarily due to respiratory evaporation) during severe heat stress is a significant demand on energy and therefore a reduced energy requirement or more efficient energy use would assist sheep in coping with heat stress. Supplementation feed additives with yeast and/or betaine have been shown to provide some benefits to poultry and cattle during periods of heat stress (Cronje 2005; Huber *et al.* 1994; Shane 2003; Wiedmeier *et al.* 1987). Given the similarities in the digestive function of sheep and cattle, the finding of increased growth rates of the sheep in the current study is consistent with results in cattle studies.

Higher daily intakes of water at 30°C and 40°C compared to 20°C by sheep and their preference for drinking water at 30°C over 20°C in hot conditions suggest that the provision of chilled water to sheep, as a heat stress management strategy, may not be beneficial. It is a widely held view that inanition of Merino sheep during the summer months in the Middle East is largely a consequence of a refusal to drink sufficient water which, during the summer months reaches temperatures of between 34°C and 43°C. This study refutes this belief, and begs questions about the value of chilling drinking water for sheep to address the problem of inanition.

Water intake recorded in these two experiments was differing. Water intake did not increase with ambient temperature in experiment one but it did increase in experiment two. This was caused by the difference of the method how the water was offered. In experiment one, water was not freely offered while in experiment two, water was offered freely. So it can be stated that water intake in experiment one was more affected by the limitation of the methodology applied, while water intake in experiment two was more affected by the animal behaviour related to the higher ambient temperature.

The mechanisms by which the yeast-based feed additive increased growth rates and the impact of increased water consumption (of warm water) on heat loss in Merino sheep were not measured in this study and there is no information available on this issue in the current literature. Studies providing an improved understanding of these responses would be valuable steps toward improving in the management of Merino sheep exposed to heat stress.

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