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Metabolic and hormonal acclimation to heat stress in domesticated ruminants

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Environmentally induced periods of heat stress decrease productivity with devastating economic consequences to global animal agriculture. Heat stress can be defined as a physiological condition when the core body temperature of a given species exceeds its range specified for normal activity, which results from a total heat load (internal production and environment) exceeding the capacity for heat dissipation and this prompts physiological and behavioral responses to reduce the strain. The ability of ruminants to regulate body temperature is species- and breed-dependent. Dairy breeds are typically more sensitive to heat stress than meat breeds, and higher-producing animals are more susceptible to heat stress because they generate more metabolic heat. During heat stress, ruminants, like other homeothermic animals, increase avenues of heat loss and reduce heat production in an attempt to maintain euthermia. The immediate responses to heat load are increased respiration rates, decreased feed intake and increased water intake. Acclimatization is a process by which animals adapt to environmental conditions and engage behavioral, hormonal and metabolic changes that are characteristics of either acclimatory homeostasis or homeorhetic mechanisms used by the animals to survive in a new 'physiological state'. For example, alterations in the hormonal profile are mainly characterized by a decline and increase in anabolic and catabolic hormones, respectively. The response to heat load and the heat-induced change in homeorhetic modifiers alters post-absorptive energy, lipid and protein metabolism, impairs liver function, causes oxidative stress, jeopardizes the immune response and decreases reproductive performance. These physiological modifications alter nutrient partitioning and may prevent heat-stressed lactating cows from recruiting glucose-sparing mechanisms (despite the reduced nutrient intake). This might explain, in large part, why decreased feed intake only accounts for a minor portion of the reduced milk yield from environmentally induced hyperthermic cows. How these metabolic changes are initiated and regulated is not known. It also remains unclear how these changes differ between short-term v. *long-term heat acclimation to impact animal productivity and well-being. A better understanding of the adaptations enlisted by ruminants during heat stress is necessary to enhance the likelihood of developing strategies to simultaneously improve heat tolerance and increase productivity.*

Keywords: ruminants, heat stress, metabolism, acclimation, adaptation

Implications

Heat stress is a significant financial burden to animal agriculture in most areas of the world. Acclimation to heat stress imposes behavioral, physiological and metabolic adjustments to reduce the strain and enhances the likelihood of surviving the stress, and it also frequently reduces ruminant performance and compromises health. Improving our knowledge of physiological and metabolic mechanisms of acclimation may contribute to the development and adoption of procedures (genetic, managerial and nutritional) that may help to maintain health, reproductive and productive efficiency in high-yielding ruminants living in hot environments. There is evidence of genetic differences within ruminants with respect to heat tolerance and this may provide clues or tools to select productive and thermotolerant subjects.

Introduction

Climate change, defined as the long-term misbalance of customary weather conditions such as temperature, wind and rainfall characteristics of a specific region, is likely to be one of the main challenges that mankind faces during the present century. The earth's climate has warmed in the last century (0.74 ± 0.18°C) with the 1990s and 2000s being the warmest on instrumental record (Intergovernmental Panel

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on Climate Change (IPCC), 2007). Furthermore, the earth's climate is predicted to continually change at rates unprecedented in recent human history (IPCC, 2007). Current climate models indicate a 0.2°C increase per decade for the next two decades and predict the increase in global average surface temperature by 2100 may be between 1.8°C and 4.0°C (IPCC, 2007).

The increasing concern with the thermal comfort of agricultural animals is justifiable not only for countries occupying tropical zones, but also for nations in temperate zones in which high ambient temperatures are becoming an issue (Nardone *et al.*, 2010). In terms of adaptation measures, it is generally faster to improve welfare, production and reproduction performances by altering the environment (West, 2003; Mader *et al.*, 2006). However, intense environmental modification (i.e. air conditioning) may be too expensive, economically unjustifiable or unsustainable. Therefore, a more consistent food supply for consumers and economic advantages to producers exist if improved thermotolerance could be accomplished without adversely affecting production (Collier *et al.*, 2005).

Heat stress negatively influences farm animal productivity and thus it both jeopardizes the human food supply chain and the livestock economy. Advances in management strategies in part alleviate the impact of thermal stress on animal performance during the hotter seasons. However, the negative effects of heat stress will become more apparent in the future if climate change continues as most predicted and as the world's population and food supply continue to increase in, and migrate toward the tropical and subtropical regions, respectively (Roush, 1994). In addition, genetic improvement programs that enhance production traits may increase an animal's susceptibility to high environmental temperatures due to the close relationship between metabolic heat generation and production level (Kadzere *et al.*, 2002).

Homeothermic animals (depending on their physiological state) have a thermoneutral zone where energy expenditure to maintain normal body temperature is minimal, constant and independent of environmental temperature (Yousef, 1987). When environmental variables, such as ambient temperature, humidity, air movement and solar radiation combine with reach values that surpass the upper limit of the thermoneutral zone, animals enter a condition known as heat stress. Heat stress occurs when the core body temperature of a given species exceeds its range specified for normal activity resulting from a total heat load (internal production and environment) exceeding the capacity for heat dissipation. This prompts physiological and behavioral responses to reduce the strain. Behavioral and physiological responses are initiated to increase heat loss and reduce heat production in an attempt to maintain body temperature within the range of normality. Initial responses are considered homeostatic mechanisms and include increased water intake, sweating and respiration rates, reduced heart rate and feed intake (Yousef, 1985; Horowitz, 2002). If exposure to the thermal load is prolonged, heat acclimation (if survivable) is achieved via processes of acclimatory homeostasis (Horowitz, 2002), and these are partially characterized by a decrease in growth hormone (GH), catecholamine and glucocorticoid levels. This altered endocrine status acts to lower circulating levels of thyroxine (T4) and triiodothyronine (T3), and this reduces the basal metabolic rate and thus heat production (Johnson, 1980; Yousef, 1987).

The objectives of this review are to focus and discuss how ruminants physiologically and metabolically acclimate to heat stress. The preponderance of information and thermal biology literature pertains to cattle, compared with other dairy ruminant species (primarily sheep, goats and buffaloes), and therefore the majority of data reviewed and discussed herein are concentrated on cattle. Bioclimatic indices and their accuracy in measuring heat stress, and biological consequences of heat stress on ruminant health, production and reproduction are reviewed.

Heat stress, critical temperatures and bioclimatic indices

The estimation of how 'comfortable' or 'stressful' environmental conditions are is complicated. This is mainly due to the various combinations of factors such as temperature, humidity, wind and direct and indirect radiations. A plethora of biometeorological indices has been developed and these empirical formulas would ideally predict the weather conditions when ruminants start to experience heat stress (Bohmanova *et al.*, 2007) and become susceptible to heat-induced death (Vitali *et al.*, 2009). Most temperature–humidity indices (THIs) are a combination of only ambient temperature (often referred to as dry-bulb temperature) and relative humidity. These two variables are easily measured and often made publically available from meteorological services. The primary difference among most THI equations is how much emphasis (weight) is placed on relative humidity and thus different equations will be better suited for different geographic locations (Bohmanova *et al.*, 2007). Although solar radiation can be a strong contributor to heat load, it is often not easily measurable and its effect is partially dependent on animal coat characteristics. Therefore, THI equations that do not incorporate a wet bulb variable (a measure of temperature, humidity, wind and solar radiation) have limitations for pasture- and feedlot-based systems.

The Livestock Conservation Institute originally arbitrarily categorized varying THI values into mild, moderate and severe for lactating cattle (Armstrong, 1994) based upon the retrospective analysis from studies conducted in the 1950s and 1960s. It was traditionally thought that milk synthesis begins to decrease when the THI reaches 72 (Armstrong, 1994), but recent data from the University of Arizona indicate that high-yielding dairy cows reduce milk yield at a THI of approximately 68 (Zimbelman *et al.*, 2009). As long as genetic selection continues to be primarily based on annual milk yield, cows will likely continue to become more susceptible to heat stress conditions.

Acclimation and adaptation to heat stress

Among the physical environmental stressors, ambient temperature is ecologically the most important (Horowitz, 2002).

Table 1 *Some endocrine adaptation made during heat acclimation in cattle*

Tissue	Response	Reference
Adrenal	Reduced aldosterone secretion	Collier *et al.* (1982a)
	Reduced glucocorticoid secretion	Collier *et al.* (1982b)
		Ronchi *et al.* (2001)
	Increased epinephrine secretion	Alvarez and Johnson (1973)
	Increased progesterone secretion	Collier *et al.* (1982b)
		Ronchi *et al.* (2001)
Adipose tissue	Increased leptin secretion	Bernabucci *et al.* (2006)
Pituitary	Increased prolactin secretion	Wetteman and Tucker (1979)
		Ronchi *et al.* (2001)
	Decreased somatotropin secretion	McGuire *et al.* (1991)
Thyroid	Decreased thyroxine secretion	Collier *et al.* (1982b)
		Nardone *et al.* (1997)
Placenta	Decreased estrone sulfate secretion	Collier *et al.* (1982b)

The terms for describing how animals respond to thermal challenges have been defined by the International Commission for Thermal Physiology (International Commission for Thermal Physiology (ICTP), 2001). This section will define and review some terms of thermal biology before a discussion of the interrelationships between ruminants and heat stress.

Acclimation: Physiological or behavioral changes occurring within the lifetime of an organism, which reduce the strain or enhance strain endurance. Strain is described as experimentally induced stressful changes in particular climatic factors such as ambient temperature in a controlled environment.

Acclimatization: Physiological or behavioral changes occurring within the lifetime of an organism in response to changes in its natural climate (e.g. seasonal or geographical).

Adaptation: Changes that reduce the physiological strain produced by stressful components of the total environment. This change may occur within an organism's lifetime (phenotypic) or be the result of genetic selection in a species or subspecies (genotypic). Acclimation, as defined by the International Commission for Thermal Physiology (ICTP, 2001), relates to *phenotypic adaptations* to specified climatic components. In thermal physiology, using the term adaptation does not require specification of the climatic components of the total environment to which the organism adapts, but the most obvious component is often denoted (e.g. adaptation to heat). There are no distinct terms that relate genotypic adaptations to the climate or particular components of climate. In comparison to adaptation as defined in neurophysiology, the adaptive processes in thermal physiology usually occur with larger time constants.

Adaptation, genotypic: A genetically fixed condition of a species or subspecies (or its evolution), which favors survival in a particular total environment.

Adaptation, phenotypic: Changes that reduce the physiological and/or emotional strain produced by stressful components of the total environment and occurring within the lifetime of the organism (synonym: adaptation non-genetic).

In this review, we will use the term *acclimation*, but the content applies equally to *acclimatization* or *phenotypic adaptation*.

Acclimation involves an altered expression of pre-existing features and is a process driven by the endocrine system (Table 1), with the goal of maintaining animal well-being regardless of environmental challenges. The long-term acclimation to heat was classically referred to as acclimatory homeostasis (Horowitz, 2002), but has recently been proposed to be a homeorhetic mechanism (Collier *et al.*, 2005), because it appears to alter the set-points of homeostatic-related systems (i.e. basal and stimulated carbohydrate metabolism). This review uses 'homeorhesis', a Waddington term (Waddington, 1957) originally used to describe changes in homeostasis in lactating cows (Bauman and Currie, 1980), which is essentially interchangeable with 'teleophoresis' as described by Chilliard *et al.* (2000).

The precise meanings of homeorhesis and teleophoresis differ according to the Greek language; however, each term refers to the 'orchestrated or coordinated changes in the metabolism of body tissues necessary to support a physiological state' (Bauman and Currie, 1980; Chilliard *et al.*, 2000). Thus, homeorhetic–teleophoretic regulation involves a dynamic coordination of physiological processes in support of a dominant physiological state or chronic situation. The described control is characterized by its chronic nature (hours or days) *v.* an acute response (seconds or minutes) characteristic of homeostatic-regulating examples. In addition, it simultaneously influences multiple tissues and systems, which results in an overall coordinated response, and it is mediated through altered responses to homeostatic signals (Bauman and Currie, 1980; Chilliard *et al.*, 2000).

Acclimation is a process that takes days to several weeks to occur and involves changes in hormonal signals that affect target tissue responsiveness to environmental stimuli. Therefore, a close examination of this process reveals that it resembles homeorhetic/teleophoretic mechanisms (Collier *et al.*, 2005). This can be deduced by the functional differences between acclimatory responses and homeostatic or 'reflex responses' (Bligh, 1976). The acclimatory response needs days or weeks to occur in contrast to seconds or minutes. In addition, acclimatory responses possess a hormonal link in the pathway from the central nervous system

to the effector cells, and usually alter the response of effector cells or organs to an environmental challenge. The main effect of these acclimatory responses is to coordinate metabolism to achieve a new equilibrium that could be considered as a new physiological state.

There is evidence for a temporal biphasic pattern of heat acclimation: short-term and long-term heat acclimations (STHA and LTHA, respectively; Horowitz *et al*., 1996; Hahn, 1999; Horowitz, 2002). STHA is the phase in which changes are initiated within cellular signalling pathways (Horowitz *et al*., 1996) leading to disturbances in cellular homeostasis and begins to reprogram cells to survive the deleterious effects of heat stress (Horowitz, 2001). In rodents, the full expression of STHA is attained when the drop in plasma thyroid hormones (T3 and T4) level exceeds 30% to 40% (Horowitz, 2001). In ruminants, the STHA is characterized by responses initiated to compensate for the increased heat stress before permanent acclimation can be obtained. Increased heat dissipation (primarily through evaporative heat loss), reduced feed intake and milk yield and increased water intake are examples of the STHA response (Gaughan *et al*., 2009a).

When the initial acclimation phase is complete and the heat-acclimated phenotype is expressed, LTHA occurs (Horowitz, 2001 and 2002). LTHA is characterized by a reprogrammed gene expression and cellular response resulting in enhanced efficiency of signalling pathways and metabolic processes (Horowitz, 2002), and this, in large part, may be mediated by heat shock proteins (Hsp; described later; Maloyan *et al*., 1999). This phase of acclimation is also characterized by endocrine changes and this is presumably with the goal of decreasing metabolic heat production and increasing heat dissipation. Two examples of LTHA are (i) chickens hatched from eggs incubated at elevated temperatures are more tolerant to heat (Yalçin *et al*., 2008a and 2008b) and (ii) body temperatures from chronically (40 to 45 days) heat-stressed (82 THI) ewes gradually return to pre-stress values (65 THI; Figure 1) and similar results have been reported for heifers (Bernabucci *et al*., 1999) and cattle (Hahn, 1999).

Evidence suggests that within domesticated ruminants differences exist between species, breed and production level with regard to heat stress susceptibility (Silanikove, 2000b; Kadzere *et al*., 2002; Collier *et al*., 2005). This is mainly due to species differences in the ability to reduce metabolic and endogenous heat production and increase heat dissipation. For instance, animals adapted to hot environments have lower metabolic and water turnover rates, and a higher capacity to dissipate heat via panting and sweating (Gaughan *et al*., 2009a). Among species, sheep and goats are considered less sensitive to heat stress than cattle (Silanikove, 2000a and 2000b; Kalifa *et al*., 2005). Silanikove (2000b) reported that goats are the best adapted species to harsh environments and goats indigenous to harsh environments perform better than other domesticated ruminants (Shkolnik and Silanikove, 1981; Devendra, 1990). The lower basal heat production, larger salivary glands and thus saliva secretion, higher surface area of absorptive mucosa, increased efficiency in the ability to recycle urea from blood to the rumen (compared to other grass and roughage eaters) and a capacity to substantially increase the volume of the foregut when fed high fibrous feed are the main morpho-physiological characteristics that allow goats to more easily adapt to harsh environments.

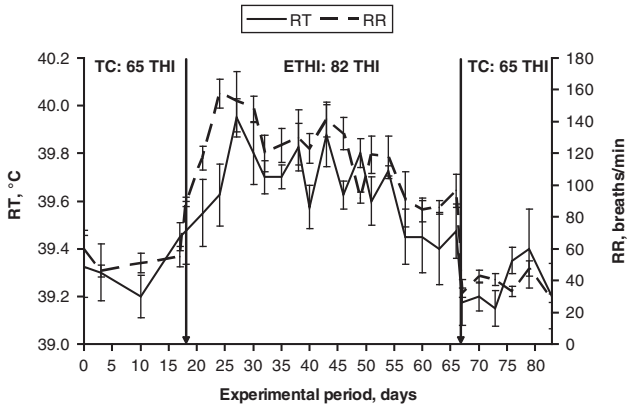

Figure 1 Least square means (±s.e.) of rectal temperature (RT) and respiration rate (RR) of sheep exposed to thermal comfort (TC; 65 THI) and elevated temperature–humidity index (ETHI; 82 THI). RT was registered at 0900 h using a digital thermometer with 0.1°C accuracy of measurement. RR was taken before the registration of RT, by counting flank movements for 1 min. Measurements were performed in duplicate (Bernabucci *et al*., 2009).

The increased susceptibility of cattle to heat stress is primarily due to their high metabolic rate, compared with that of other ruminants, and the poorly developed water retention mechanism in the kidney and gut. For example, the rate of water use by heat-stressed Holstein heifers was 2.84-fold higher (0.56 *v.* 0.20 l/kg$^{0.75}$ body weight (BW)) than heat-stressed Sardinian female lambs (Bernabucci *et al*., 1999 and 2009). In addition, beef cattle are less sensitive than dairy cattle to heat stress due to the overall decrease in endogenous heat production (lower plane of production, reduced heat increment of feeding, etc.). Furthermore, Senft and Rittenhouse (1985) proposed a model to evaluate thermal acclimation in cattle, which clearly demonstrated significant variability between beef cattle breeds. The variability was in the length of acclimation period and ranged from 9 days for Angus and Charolais to 14 days for Polled Hereford (Hereford and Santa Gertrudis had intermediate values (12 days)). In a recent study, Brown-Brandl *et al*. (2006) reported a significant effect of breed and breed by temperature interaction on respiration rates and panting scores. Angus and MARC III breeds had the highest respiration rate and panting score, followed by Gelbvieh and then Charolais. Those authors concluded that the results seemed logically due to hide color differences that affect the adsorption of solar radiation.

The level of acclimation to heat stress also varies among breeds within the same species and this is probably because *Bos indicus* and *Bos taurus* cattle have evolved within distinct climates (Hansen, 2004). During genetic adaptation, Zebu cattle have acquired thermotolerant genes

(Hansen, 2004), and therefore have a higher degree of thermotolerance compared with *B. taurus* species. Gaughan *et al*. (1999) reported that genetic adaptation allows *B. indicus* cattle to have lower respiration rates and rectal temperatures than *B. taurus* species when exposed to similar heat stress conditions. In terms of milk yield, Jersey dairy cows are more resistant to heat stress than Holstein cows (Sharma *et al*., 1983), and Ragsdale *et al*. (1953) reported that milk production of Holstein, Jersey and Brown Swiss cows exposed to 34°C was 63%, 68% and 84%, respectively, of thermal neutral conditions. However, the degree of 'thermotolerance' depends upon which variable is used to evaluate acclimation. For example, *in vitro* data indicate that lymphocyte tolerance to heat was lower in Brown Swiss compared with Holstein cows and this was associated with increased *Hsp72* expression (Lacetera *et al*., 2006).

Acclimation responses also depend on pre-stress production levels. For example, low-yielding lactating cows are able to return to pre-heat stress production levels but high-producing cows cannot (Johnson and Vanjonack, 1976), and this may be because the zone of thermal neutrality shifts to lower temperatures, as milk yield, feed intake and metabolic heat production increase (Coppock *et al*., 1982; Kadzere *et al*., 2002). In addition, the slope/persistency of milk yield decline during heat stress is steeper (−0.059%/day) in higher-producing (30 kg/day) cows than in lower (25 kg/day)-producing cows (−0.019%/day; Johnson *et al*., 1987). In fact, increasing daily milk yield from 35 to 45 l is thought to increase sensitivity to thermal stress and reduces the 'threshold temperature' by 5°C (Berman, 2005). This increased sensitivity is presumably due to the extra heat associated with synthesizing additional milk. For example, heat production from cows producing 18.5 and 31.6 kg milk/day was 27.3% and 48.5%, respectively, higher than non-lactating cows (Purwanto *et al*., 1990). Moreover, a cow weighing 700 kg and yielding 60 kg milk/day produces about 44 171 kcal of heat/day; the same cow produces 25 782 kcal of heat/day at the end of lactation (milk yield of 20 kg/day) (Nardone *et al*., 2006).

Genomic responses during acclimation

As reported above, high-producing dairy ruminants are acutely susceptible to environmentally induced hyperthermia because the metabolic heat load is proportionate to milk production levels. To counteract an increasing heat load, dairy cows must enlist adaptations on a cellular basis to increase heat dissipation and minimize heat production. However, the genomic alterations and associated molecular mechanisms leading to cellular heat stress acclimation are poorly understood. Recently, studies aimed at characterizing the global changes in cellular gene expression in cattle have incorporated microarray analysis utilizing bovine-specific cDNA arrays obtained from the National Bovine Functional Genomics Consortium. Microarray analysis profiled bovine mammary epithelial cell (BMEC) gene expression in response to acute heat stress using an *in vitro* system that approximates mammary development and function (Collier *et al*., 2008). During severe and acute hyperthermia (42°C), the BMEC exhibited morphological changes (regression of ductal branches) and reduced cellular growth. Consistent with these physical alterations in cell behavior, gene expression associated with protein synthesis and cellular metabolism was decreased. In this model system, *Hsp70* gene expression in BMEC remained elevated for 4 h at 42°C and then returned to basal levels after 8 h of exposure, indicating the end of heat tolerance and activation of genes associated with apoptosis (Collier *et al*., 2008). A second study in lactating dairy cattle evaluated the gene expression profile of liver tissue in response to an extended period (14 days) of the heat stress (Rhoads *et al*., 2005). The liver's pivotal role in whole-body metabolism via coordination of endogenously and exogenously derived nutrients is most likely altered by heat stress-induced reductions in feed intake and shifts in metabolism. In agreement with this notion of hepatic remodelling, liver gene expression favoured reductions in cell growth and proliferation, and also in enhanced apoptosis.

A large proportion of an animal's mass comprises skeletal muscle, which can have a profound impact on whole-animal energy metabolism and nutrient homeostasis, especially during periods of stress. To better understand how an environmental heat load influences the set points of several metabolic pathways within skeletal muscle, Rhoads *et al*. (2008) examined heat stress effects on skeletal muscle during beef cattle adaptation to chronic heat stress using microarray analysis. Skeletal muscle (semimembranosus) biopsies were obtained during thermal neutral conditions and again after exposure to heat stress. Data interrogation by pathway analysis identified dramatic changes in the skeletal muscle transcriptional profile revealing that during heat stress bovine skeletal muscle may experience mitochondrial dysfunction leading to impaired cellular energy status. This may have broad implications for the reduced growth, decreased milk production and heat intolerance observed in ruminants during heat stress especially if skeletal muscle is not able to make necessary contributions to whole-body energy homeostasis. Taken together, the microarray data demonstrate that bovine cells and tissues undergo changes in cellular behavior, which may be important for individual tissue function, whole-body metabolism and overall physiological acclimation to heat stress.

Metabolic and hormonal acclimation to hot environment

The biological mechanism by which heat stress impacts production and reproduction is partly explained by reduced feed intake, but also includes an altered endocrine status, reduction in rumination and nutrient absorption, and increased maintenance requirements (Collier *et al*., 2005), resulting in a net decrease in nutrient/energy availability. Naturally, a reduction in energy intake combined with increased energy expenditure for maintenance lowers energy balance, and partially explains why lactating cattle lose significant amounts of BW during severe heat stress (Rhoads *et al*., 2009; Shwartz *et al*., 2009).

Reductions in energy intake coupled with increased maintenance costs during heat stress causes negative energy balance (NEBAL) in lactating cows (likely stage of lactation independent) and a bioenergetic state, similar (but not to the same extent) to the NEBAL observed in early lactation. The NEBAL associated with the early *post-partum* period is coupled with increased risk of metabolic disorders and health problems, decreased milk yield and reduced reproductive performance (Drackley, 1999). Similarly, we hypothesize many of the negative effects of heat stress on production, and animal health and reproduction indices may be mediated by NEBAL. However, it is not clear how much of the reduction in performance (yield and reproduction) can be attributed or accounted directly (hyperthermia) or indirectly (reduced feed intake) to heat stress.

As stated above, heat stress acclimation is accomplished by changes in homeostatic responses (Horowitz, 2002) and may include homeorhetic/teleophoretic processes involving an altered endocrine status that ultimately affects target tissue responsiveness to environmental stimuli. Hormones are also implicated in the acclimatory response to heat stress (Collier *et al*., 2005), and they primarily include thyroid hormones, prolactin, GH, glucocorticoids and mineralcorticoids. The thyroid hormones, T4 and T3, provide a major mechanism important for acclimation and have received considerable research attention. It is well known that heat acclimation decreases endogenous levels of thyroid hormones (in an attempt to reduce endogenous heat production) and those mammals adapted to warmer climates follow this pattern (Johnson and Vanjonack, 1976; Horowitz, 2001).

Circulating prolactin levels are increased during thermal stress in a variety of mammals including ruminants (Collier *et al*., 1982a; Ronchi *et al*., 2001; Roy and Prakash, 2007). This is paradoxical as reduced nutrient intake in thermal neutral ruminants decreases circulating prolactin concentrations (Bocquier *et al*., 1998). A direct (independent of reduced feed intake) effect of heat stress on serum prolactin levels has been shown (Ronchi *et al*., 2001). Increased prolactin initially seems contradictory (especially in lactating dairy cows), given prolactin's well-known role in maintaining galactopoiesis in some species and lactogenesis in ruminants, but prolactin may play an important role in acclimation through improved insensible heat loss and sweat gland function (Beede and Collier, 1986). Bromocryptine (a prolactin secretion inhibitor) treatment during heat acclimation affects sweat gland function by preventing increased sweat gland discharge (Kaufman *et al*., 1988). Prolactin levels also differ with season and it appears to be involved in acclimating to seasonal/weather changes (Leining *et al*., 1979). Whether increased prolactin levels affect the ability of animals to metabolically adapt during a heat load is currently unknown but is of interest, given its importance as a homeorhetic hormone.

The hypothalamic–pituitary–adrenal axis is also a key component of the acclimatory response to thermal stress (Beede and Collier, 1986; Collier *et al*., 2005). Corticotropin-releasing hormone stimulates somatostatin; possibly a key mechanism by which heat-stressed animals have reduced GH and thyroid levels (Riedel *et al*., 1998). In dairy cattle, the glucocorticoids decrease during acclimation at 35°C (Alvarez and Johnson, 1973) and are lower in thermal-acclimated animals compared with controls (Ronchi *et al*., 2001).

A prerequisite of understanding the metabolic adaptations that occur during heat stress is an appreciation of the physiological and metabolic adaptations to thermal neutral NEBAL (i.e. malnourishment or during the transition period). NEBAL is associated with a variety of metabolic changes that are implemented to support the dominant physiological condition of lactation (Bauman and Currie, 1980). Marked alterations in both carbohydrate and lipid metabolism ensure partitioning of dietary-derived and tissue-originating nutrients toward the mammary gland, and not surprisingly many of these changes are mediated by endogenous GH, which is naturally increased during periods of NEBAL (Bauman and Currie, 1980). One characteristic response to NEBAL is a reduction in circulating insulin coupled with a reduction in systemic insulin sensitivity. The reduction in insulin action allows for adipose lipolysis and mobilization of non-esterified fatty acids (NEFAs; Bauman and Currie, 1980). Increased circulating NEFAs are typical in 'transitioning' cows and represent (along with NEFA-derived ketones) a significant source of energy (and are precursors for milk fat synthesis) for cows in NEBAL. Post-absorptive carbohydrate metabolism is also altered by the reduced insulin action during NEBAL with the net effect of reduced glucose uptake by systemic tissues (i.e. muscle and adipose). The reduced nutrient uptake coupled with the net release of nutrients (i.e. amino acids and NEFAs) by systemic tissues are key mechanisms implemented by cows in NEBAL to support lactation (Bauman and Currie, 1980). The thermal neutral cow in NEBAL is metabolically flexible, in that alternative fuels (NEFAs and ketones) can be burned to spare glucose, which can be utilized by the mammary gland to copiously produce milk.

We recently showed that despite the reduced feed intake, heat-stressed cows do not have an increase in plasma NEFA (Rhoads *et al*., 2009; Shwartz *et al*., 2009), and this agrees with other heat-stressed ruminant models (Sano *et al*., 1983; Itoh *et al*., 1998; Ronchi *et al*., 1999). The lack of an elevated NEFA response is especially surprising, as acute heat stress causes a marked increase in circulating cortisol, norepinephrine and epinephrine levels (Collier *et al*., 2005), catabolic signals that normally stimulate lipolysis and adipose mobilization. This is also surprising as calculated EBAL is traditionally thought to be closely associated with circulating NEFA levels. The fact that heat-stressed cows fail to enlist this 'shift' in post-absorptive energetic metabolism (despite inadequate nutrient intake) may indicate that heat stress directly (not mediated by feed intake) impacts energetics.

An additional metabolic hallmark of malnourished animals in thermal neutral conditions is either a reduction in blood insulin or a decrease in systemic insulin sensitivity (Bauman and Currie, 1980). Despite marked reductions in nutrient intake, heat-stressed cattle exhibit increased basal insulin levels and stimulated insulin response (Itoh *et al*., 1998;

Baumgard and Rhoads, 2007; Wheelock *et al*., 2010), and this agrees with heat-stressed rodent experiments (Torlinska *et al*., 1987). The increased basal and stimulated insulin levels may explain the lack of an increase in basal NEFA levels in heat-stressed cows as insulin is a potent antilipolytic hormone (Vernon, 1992). The increase in basal insulin levels appears due to increased pancreas secretion, rather than reduced circulating insulin removal, because of the acute marked difference in insulin levels between heat-stressed and thermal neutral pair-fed cows following administration of an insulin secretagogue (Baumgard and Rhoads, 2007).

In addition to adipose tissue, skeletal muscle is also mobilized during the periods of inadequate nutrient intake (in thermal neutral conditions) to support lactation. We have shown that heat-stressed cows (Baumgard and Rhoads, 2007; Shwartz *et al*., 2009) and heifers (Ronchi *et al*., 1999) have increased plasma urea nitrogen levels compared with the thermal neutral controls. Plasma urea nitrogen can originate from at least two sources: inefficient rumen ammonia incorporation into microbial proteins or from hepatic deamination of amino acids mobilized from the skeletal muscle. A better circulating indicator of muscle catabolism is either 3-methyl-histidine or creatine, both of which are increased in heat-stressed poultry (Yunianto *et al*., 1997), rabbits (Marder *et al*., 1990) and lactating cows (Schneider *et al*., 1988). Additional evidence suggesting that heat stress alters protein metabolism is decreased milk protein levels from heat-stressed cows (Rhoads *et al*., 2009; Shwartz *et al*., 2009), and it appears that α_S- and β-casein synthesis is most susceptible (Bernabucci *et al*., 2002b). The effects of heat stress on muscle and mammary protein metabolism is perplexing, as insulin typically stimulates protein synthesis in both tissues.

Cellular acclimation during hyperthermia involves the coordination of cellular responsiveness to endocrine cues coupled with intrinsic changes, a concept best illustrated in recent studies examining the somatotropic axis (Rhoads *et al*., 2009, 2010). Dairy cattle in positive energy balance have a coupled somatotropic axis, defined as efficient GH-dependent insulin-like growth factor-I (IGF-I) synthesis and secretion by the liver in response to pituitary-derived or exogenous GH (Bauman and Vernon, 1993). However, during the periods of NEBAL, circulating GH concentrations rise and plasma IGF-I concentrations fall, and the response of hepatic IGF-I production to exogenous GH is negligible at best (Bauman and Vernon, 1993). These observations indicate that the somatotropic axis becomes uncoupled during the periods of NEBAL (Bauman and Vernon, 1993).

Some have reported numerical or statistically significant reductions in GH levels during thermal stress (Mitra *et al*., 1972; McGuire *et al*., 1991). However, we have recently reported no differences in mean GH concentrations, GH pulsatility characteristics or GH response to GH-releasing factor in heat-stressed *v.* pair-fed thermal neutral controls (Rhoads *et al*., 2009). Despite these similar aspects of circulating GH, plasma IGF-I was reduced in heat-stressed cows. Circulating IGF-I has been implicated in the regulation of milk synthesis in dairy cattle and blood-borne IGF-I is produced in a GH-dependent fashion by the liver (Boisclair *et al*., 2006). We investigated whether hepatic GH responsiveness was altered during heat stress by measuring GH receptor abundance and STAT-5 phosphorylation (Rhoads *et al*., 2010). Heat stress, independent of reduced feed intake, decreased the abundance of the hepatic GH receptor, although both heat stress and malnutrition were sufficient to decrease STAT-5 phosphorylation. Consistent with reduced GH signalling through STAT-5, hepatic IGF-I mRNA abundance was lower in heat-stressed animals. Thus, the reduced hepatic GH responsiveness observed during heat stress appears to involve mechanisms independent of reduced feed intake. The physiological significance of reduced hepatic GH receptor abundance during heat stress is unclear at this time, but may serve to alter other GH-dependent hepatic processes such as regulation of gluconeogenesis.

Water metabolism

Increased water loss via skin and respiratory evaporation (sweating and panting, respectively) in an attempt to dissipate heat can disturb body water levels and mineral concentrations, particularly within vascular and extracellular compartments. The altered heat-induced changes in both circulating water and minerals interfere with the animal's ability to maintain proper osmotic balance and blood pressure. In fact, Silanikove (1994) reported that increased body fluid loss due to sweating and panting in heat-stressed ruminants can increase the risk of cardiovascular dysfunction and an inability to maintain euthermia.

Heat-stressed high-producing dairy cows may have problems maintaining a steady plasma volume. However, in our controlled environment study (Nardone *et al*., 1992), we reported a positive correlation ($r = 0.62$; $P < 0.01$) between water intake and rectal temperature in heat-stressed lactating cows. In addition, Silanikove (1992) reported that heat stress influences water metabolism by increasing plasma and extracellular fluid volume in proportion to the thermoregulatory requirement of the cow. Kadzere *et al*. (2002) suggested that either the increased efficiency of water transfer through the biological membranes ($\sim 50\%$) or increased plasma volume provides a thermoregulatory advantage.

Biological consequences of hormonal and metabolic acclimation

Animals initiate many acute acclimation responses in order to survive thermal stress (Stott, 1981), but some of these adaptations may ultimately adversely affect long-term health and/or productivity. In the sections below, we will address how physiological and metabolic adjustments affect health variables and production parameters.

Health

It is assumed that high ambient temperatures directly and indirectly affect the health status of farm animals (Gaughan *et al*., 2009a). Direct influences include temperature-related

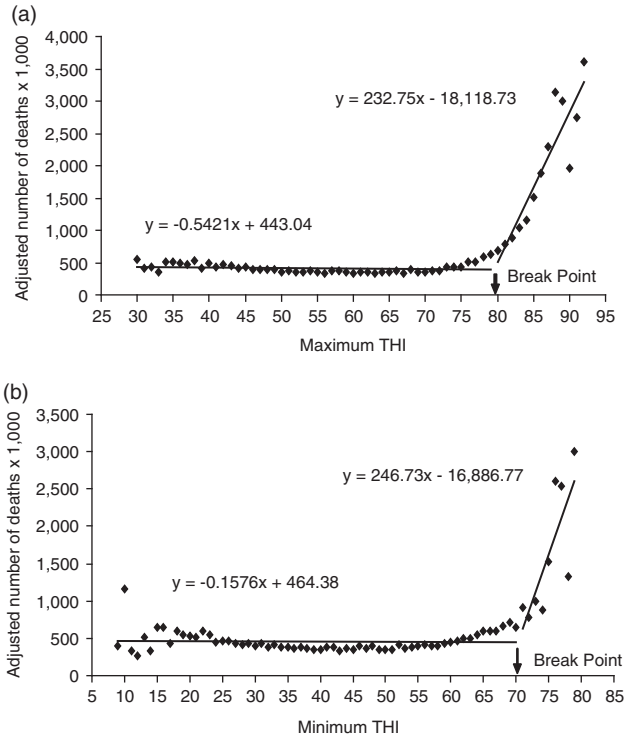

Figure 2 Relationship between daily temperature–humidity index (THI) and mortality. Adjusted number of deaths (ANDs) in relation to maximum (a) and minimum (b) THI (Vitali *et al.*, 2009). (a) A break point was detected at 79.6 THI. Below the break point, the AND was constant across THI values ($R^2 = 0.0119$, $F_{1,50} = 0.910$, $P = 0.5$), whereas above 79.6 THI, the AND rose sharply with THI ($R^2 = 0.8382$, $F_{1,13} = 269.65$, $P < 0.001$). (b) A break point was detected at 70.3 THI. Below the break point, the AND was constant across THI values ($R^2 = 0.0004$, $F_{1,62} = 0.930$, $P = 0.5$), whereas above 70.3 THI, the AND rose sharply with THI ($R^2 = 0.6151$, $F_{1,9} = 707.01$, $P < 0.001$).

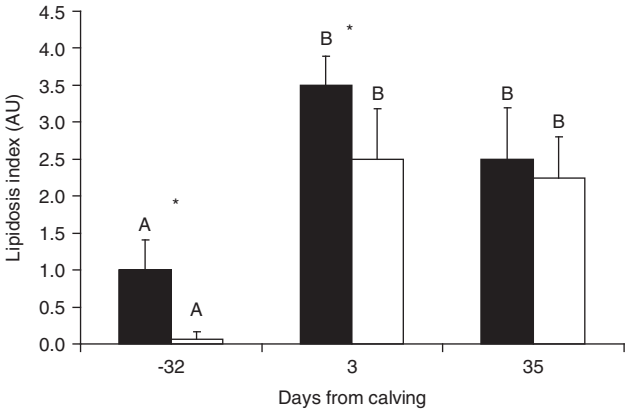

Figure 3 Least square means and s.e. for the degree of liver lipidosis in transition dairy cows during summer (■) and spring (□); $^*P < 0.05$ within day; A, B = $P < 0.01$ within season (adapted from Basiricò *et al.* (2009)).

illness and death. Indirect influences include those derived from reduced nutrient intake, altered microbial populations (around and in the animal), redistribution of vector-borne diseases, decreased host resistance to infections, water shortages and food-borne diseases. A series of studies have described a higher risk of mortality during the hottest months (Dechow and Goodling, 2008; Vitali *et al.*, 2009), and an increased death rate during extreme weather events (Hahn *et al.*, 2002). High temperatures may cause heat stroke, heat exhaustion, heat syncope, heat cramps and ultimately organ dysfunction, and these heat-induced complications occur when the body temperature rises approximately 3°C to 4°C. Our recent epidemiological dairy cow study (Vitali *et al.*, 2009) indicates that 80 and 70 are the daily maximum and minimum THI values, respectively, above which heat-induced death rate increases. In addition, 87 and 77 are the daily upper critical maximum and minimum THI, respectively, above which the risk of heat-induced death becomes maximum (Figure 2).

Organisms initiating the stress acclimation process make a trade-off between 'survival' and an effective immune system, and it appears that heat shock factors and Hsp occupy a central place in this balance (Morange, 2006). We recently reported that incubating bovine peripheral blood mononuclear cells under high temperatures is associated with both a gradual reduction in their ability to proliferate in response to mitogen stimulations and with a corresponding gradual increased *Hsp72* gene expression (Lacetera *et al.*, 2006). Furthermore, under these experimental conditions, a negative correlation was detected between DNA synthesis and *Hsp72* mRNA levels. These results indicate that in cells exposed to stressful conditions, the synthesis of 'protective' proteins predominates and the synthesis of proteins or other molecules involved in proliferation is reduced. A similar association between severe heat stress and altered lymphocyte function was observed under field conditions (Lacetera *et al.*, 2005). In the same study, we hypothesized that depressed cell-mediated immunity and an enhanced humoral response might be related to heat-induced increases in circulating cortisol by causing a shift from a T-helper 1 (Th1; cellular) to a Th2 (humoral) pattern of immunity leading to increased infection susceptibility.

Several dairy cow studies report increased occurrence of mastitis during the summer (Morse *et al.*, 1988; Waage *et al.*, 1998). Improved survival capability or multiplication of pathogens or their vectors (Chirico *et al.*, 1997), or a negative action of heat stress on defensive mechanisms (Giesecke, 1985) have been indicated as the potential causes of such epidemiological findings.

Finally, health problems in heat-stressed ruminants may also be a consequence of nutritional and metabolic acclimation. In particular, due to increased maintenance requirements for thermoregulation and lower feed intake, summer transition dairy cows are more likely to experience subclinical or clinical ketosis (Lacetera *et al.*, 1996) and are under higher risk of liver lipidosis (Figure 3; Basiricò *et al.*, 2009). Increased liver lipidosis probably compromises liver function and we have reported that heat-stressed cattle have reduced albumin secretion and liver enzyme activities (Ronchi *et al.*, 1999). We have also shown that heat stress causes oxidative stress in transition dairy cows (Bernabucci *et al.*, 2002a).

Rumen health

Heat stress has long been known to adversely affect rumen health (Mishra *et al.*, 1970) due to a variety of biological and management reasons (Bernabucci *et al.*, 1999 and 2009; Kadzere *et al.*, 2002). Heat-stressed cows consume less feed and consequently ruminate less and this results in decreased buffering agents (ruminating is the primary stimulant of saliva production) entering the rumen. In addition, because of the redistribution of blood flow to the periphery (in an attempt to enhance heat dissipation) and subsequent reduction in blood delivery to the gastrointestinal track, digestion end products (i.e. volatile fatty acids (VFAs)) are absorbed less efficiently and thus the total rumen VFA content increases (and pH decreases). Furthermore, increased respiration rates also contribute to rumen acidosis because panting causes enhanced CO_2 to be exhaled. In order to be an effective blood–pH buffering system, the body needs to maintain a 20 : 1 HCO_3^- (bicarbonate) to CO_2 ratio. Because of the hyperventilation-induced decrease in blood CO_2, the kidney secretes HCO_3^- to maintain this ratio. This reduces the amount of HCO_3^- that can be used (via saliva) to buffer and maintain a healthy rumen pH. In addition, panting cows often drool reducing the quantity of saliva available for the rumen. The reductions in saliva HCO_3^- content and the decreased amount of saliva entering the rumen make the heat-stressed cow much more susceptible to subclinical and acute rumen acidosis (Kadzere *et al.*, 2002).

Changes in cow's eating behavior probably also contribute to rumen acidosis. Cows in thermal neutral conditions typically consume 12 to 15 meals per day but decrease eating frequency to 3 to 5 meals per day during heat stress. The decreased frequency is accompanied by larger meals and thus more acid production post-eating. Furthermore, cows will typically gorge (over eat) the day following a heat wave and this gluttonous behavior is well known to cause rumen acidosis.

To compensate for the reduced nutrient and energy intake caused by heat stress and the metabolic heat load associated with fermenting forages, nutritionists typically increase the energy density of the ration using extra grains/concentrates. However, this practice should be conducted with care as this type of diet can be associated with a lower rumen pH. The combination of a 'hotter' ration and the cow's reduced ability to neutralize the rumen directly increases the risk of rumen acidosis and indirectly enhances the risk of developing negative side effects of an unhealthy rumen environment (i.e. laminitis, milk fat depression, etc.).

Reproduction

High environmental temperatures may compromise reproductive efficiency in both genders. In females, heat stress compromises oocyte growth by altering progesterone, luteinizing hormone and follicle-stimulating hormone secretion and dynamics during the estrous cycle (Ronchi *et al.*, 2001). Moreover, heat stress may reduce summer fertility in dairy and beef cows by causing poor estrous expression due to reduced estradiol secretion from the dominant follicle developed in a low luteinizing hormone environment (Biggers *et al.*, 1987; De Rensis and Scaramuzzi, 2003; Amundson *et al.*, 2006). About a 20% to 27% drop in conception rates (Lucy, 2002; Chebel *et al.*, 2004) or decrease in 90-day non-return rate to the first service in lactating dairy cows (Al-Katanani *et al.*, 1999; Ravagnolo and Misztal, 2002) occurs in summer. Roy and Prakash (2007) reported a lower plasma progesterone and higher prolactin concentration during estrous cycle in heat-stressed Murrah buffalo heifers. Those authors concluded that prolactin and progesterone profiles during the summer and winter are directly correlated with the reproductive performance of the buffalo, and that hyperprolactinemia may cause acyclicity/infertility in buffaloes during the hot summer. Heat stress has also been associated with impaired embryo development and increased embryo mortality in cattle (Wolfenson *et al.*, 2000; Bényei *et al.*, 2001; Hansen, 2007). Furthermore, heat stress during pregnancy slows down fetal growth and increases fetal loss.

In bulls, semen concentration, number of spermatozoa and motile cells per ejaculation are lower in summer than in winter and spring (Mathevon *et al.*, 1998; Nichi *et al.*, 2006). Meyerhoeffer *et al.* (1985) reported that exposure to elevated ambient temperatures resulted in decreased bull semen quality as evidenced by a reduced percentage of motile sperm, reduced sperm output and an increased percentage of abnormal and aged sperm.

Production

Milk. Johnson *et al.* (1962) showed a linear reduction in dry matter intake (DMI; −0.23 kg/day) and milk yield (−0.26 kg/day) when THI exceeded 70. In a 2-year study conducted under field conditions, we recently found a decrease of 0.27 kg of milk per THI unit only if Holstein cows were exposed to THI higher than 68 (Figure 4), and similar results were reported by Ravagnolo *et al.* (2000). Bouraoui *et al.* (2002) in a 2-year study, found a negative correlation between milk yield and daily THI ($r = -0.76$). In particular, milk yield decreased by 0.41 kg per cow per day for each THI unit increase of above 69. Bohmanova *et al.* (2007) reported different rates of milk production decline per unit of THI, ranging from −0.40 to −0.27 and from −0.59 to −0.23 kg

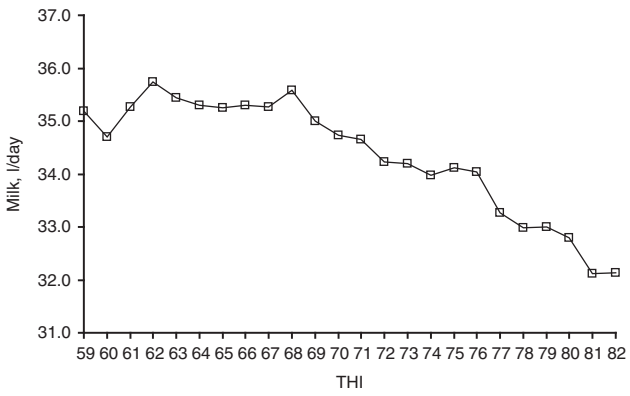

Figure 4 Temperature–humidity index on milk production in Holstein cows.

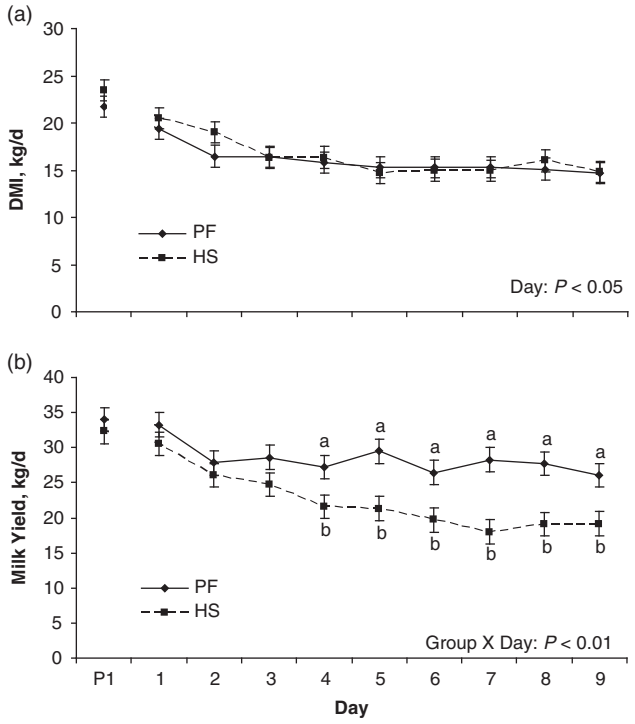

Figure 5 Effects of heat stress (HS) or pair-feeding (PF) on (a) dry matter intake and (b) milk yield in lactating Holstein cows. Solid lines with diamonds represent PF cows and dashed lines with squares represent HS cows. The mean value from days 1 to 9 of the thermal neutral *ad libitum* period (P1) was used as a covariate and is represented by P1 on the *x* axis. The results of days 1 to 9 are from P2 when cows were exposed to HS or exposed to thermal neutral conditions and PF with the HS cows (adapted from Rhoads *et al*. (2009)).

in Georgia and Arizona, respectively. Reduced feed intake caused by heat stress has traditionally been assumed to be primarily responsible for the decrease in milk yield (Collier *et al*., 1982a; West, 2003). However, we have recently shown that reduced nutrient intake (indirect effects of heat) accounts for only about 35% of the heat stress-induced decrease in milk synthesis (Figure 5).

Our studies, conducted in climatic chambers, showed a heat-induced decrease in milk yield of 35% in mid-lactating dairy cows (Nardone *et al*., 1992; Spiers *et al*., 2004; Rhoads *et al*., 2009), and 14% in early lactating dairy cows (Lacetera *et al*., 1996). Milk yield for Holstein cows declined by 0.88 kg per THI unit increase for the 2-day lag of mean THI (West *et al*., 2003). The extent of milk yield decline observed in heat-stressed cows is dependent on several factors that interact with high air temperature. As reported before, the milk yield losses during a heat load are positively correlated with production levels before heat stress. The stage of lactation is also an important factor affecting how dairy cows respond to heat stress. Johnson *et al*. (1987) observed that the mid-lactating dairy cows were the most heat sensitive compared to their early and late lactating counterparts. In fact, mid-lactating dairy cows showed a higher decline in milk production (−38%) following exposure to heat. The same results have been obtained in a field study by Calamari *et al*. (1997) who observed a decline in milk yield of 11% to 14%, 22% to 26% and 15% to 18% in early (after the first month), mid- and late lactating dairy cows, respectively.

The nutritional–metabolic conditions of dairy cows during the different lactation stages might explain the higher heat sensitivity of mid-lactating dairy cows. In fact, milk yield in early lactating cows is strongly supported by tissue stores mobilization and less by feed intake, whereas milk yield in mid-lactating cows is mostly supported by feed intake, which reaches a peak in this stage. As the metabolic utilization of tissue stores has higher efficiency compared with the metabolic utilization of feed, the early lactating cows should produce less metabolic heat per kg of milk yielded, than mid-lactating cows. This is a possible explanation why mid-lactating cows are more sensitive to hot weather.

A hot environment negatively affects milk quality as well (Moran, 1989; Bernabucci and Calamari, 1998; Calamari and Mariani, 1998). Above 72 THI, milk protein content declines, whereas the response of milk fat content seems delayed and results are contradictory. When comparing milk production during summer and spring in a dairy herd located in central Italy, we found a lower milk yield (−10%), and also lower casein percentage and casein index (casein/total proteins ratio) in summer (2.18% *v.* 2.58% and 72.4% *v.* 77.7%, respectively; Bernabucci *et al*., 2002b). The decreased casein was due to the reduction in α_s- and β-casein percentages. The strict relationship between casein content and fraction and milk behavior during the technical processes may explain the loss in cheese yield and the alteration of cheese-making properties during summer in Italy (Calamari and Mariani, 1998).

The minor importance of sheep, goats and buffalo with regard to global milk production, in addition to lower selection intensity for high productivity and their effective adaptability to hot environments, explains why less heat stress attention has been given to these species. However, reports indicate that milk production traits in ewes seem to have a higher negative correlation with the direct values of temperature or relative humidity than THI. The values of THI above which ewes start to suffer from heat stress seem to be quite different among breeds (Finocchiaro *et al*., 2005). Solar radiation seems to have a limited effect on milk yield, but a greater effect on yield of casein, fat and clot firmness in the milk of Comisana ewes (Sevi *et al*., 2001). Even goats are affected by heat stress and responded by reducing milk yield and the content of milk components (Olsson and Dahlborn, 1989).

Meat. Worldwide, beef cattle are generally reared outdoors with constant exposure to natural conditions, while maintenance in housing systems is limited. Beef cattle are particularly vulnerable not only to extreme environmental conditions but also to rapid changes in climate. In particular, fatter cattle (subcutaneous fat acts like insulation and slows heat dissipation), cattle with a heavy hair coat (more insulation) and darker-coated animals (black and dark red cattle) are very sensitive to heat (Brown-Brandl *et al*., 2006; Gaughan *et al*., 2009b). The Scientific Committee on Animal

Health and Animal Welfare (SCAHAW, 2001) suggested that the threshold temperature where adverse effects on DMI, growth and feed efficiency are readily apparent for beef cattle is 30°C with relative humidity below 80% and 27°C with relative humidity above 80% (Hahn, 1999). Mitlöhner *et al*. (2001) reported reduced DMI and average daily gain, carcass weight loss, lower fat thickness and increase in disease incidence in steers kept under heat stress conditions. Furthermore, Kadim *et al*. (2004) found strong negative effects of the hot season (average temperature of 34.3 $\pm$ 1.7°C and 48.8 $\pm$ 7.6% relative humidity) on the quality of beef meat. In particular, these authors reported higher ultimate pH values, lower Warner–Bratzler shear force and darker meat of *M. longissimus thoracis* in heat-stressed beef cattle when compared with muscle samples collected during the cool season.

Selecting heat tolerance

Finch *et al*. (1982) reported a negative correlation between sweating response and metabolic rate, which illustrates the difficulty in combining heat adaptability characteristics and production traits in cattle. Genetic selection for milk and meat production has reduced heat tolerance (Ravagnolo and Misztal, 2000; Kadzere *et al*., 2002; Gaughan *et al*., 2009a). Heat tolerance today is considered one of the most important adaptive aspects in cattle (McManus *et al*., 2009). The identification of heat-tolerant animals within high-producing breeds may be useful only if these animals are able to maintain high productivity and survivability when exposed to heat stress conditions (Gaughan *et al*., 2009a).

To establish whether it is possible to select high-producing cattle for heat tolerance, at least two important points have to be ascertained (Nardone, 1998): (i) identification of one (preferably) or more measurable indices of heat tolerance and (ii) estimation of the genetic correlation between heat tolerance and productive and reproductive traits.

The heritability of some anatomical and morphological traits (i.e. sweat glands density and function, hair coat density and thickness, hair length and color and skin color) has been reported within ruminant breeds (reviewed in Nardone, 1998). Differences in anatomical and morphological characteristics partially explain differences in heat tolerance among species and breeds (Ingram and Mount, 1975; West, 2003; Collier *et al*., 2008; Dikmen *et al*., 2009; Gaughan *et al*., 2009a and 2009b). Collier *et al*. (1981) indicated that Jersey cows were more heat tolerant that Holstein cows, and Muller and Botha (1993) suggested that part of this enhanced tolerance is the difference in respiratory rate capacity. In addition, there are obviously differences in the surface area to mass ratio between the two breeds and this could contribute to the improved heat tolerance in Jersey cows.

Sweat gland density and function, hair coat density and thickness, hair length and color, skin color and regulation of epidermal vascular supply are animal factors affecting the efficacy of evaporative heat loss. Cattle have a single apocrine sweat gland associated with each hair fiber. Thus, hair density directly affects the number of sweat glands, and hair diameter and length affect evaporative heat loss (Gebremedhin and Wu, 2001; Olson *et al*., 2006). Studies conducted at the University of Arizona indicate dairy cattle are able to maintain core body temperature until skin surface temperatures exceed 35°C (Pollard *et al*., 2005). Above this surface temperature, cows begin to store heat, rectal temperature rises, cutaneous evaporative heat loss increases and variation (in body temperature) between cows is much greater than below 35°C. Reasons for this variability are likely because of differences in the number (and activity) of sweat glands and hair coat characteristics (Olson *et al*., 2006).

Another example of thermotolerance linked with morphological characteristics is the 'slick' gene in cattle; 'Slick' cattle are characterized by shorter hair length. Cattle with shorter hair, hair of greater diameter and lighter coat color are more adapted to hot environments than those with longer hair coats and darker colors (Gaughan *et al*., 2009b). This phenotype has been characterized in *B. taurus* tropical cattle (Senepol and Carona), and this dominant gene is associated with an increased sweating rate, lower rectal temperature and lower respiration rate in homozygous cattle under hot conditions (Mariasegaram *et al*., 2007). However, no information on the association between genotype and production or reproduction traits are available. Hansen (1990) found that Holstein cows with white coat coverage greater than 70% had lower rectal temperatures compared with cows with black coat coverage greater than 70%. King *et al*. (1988) reported a positive correlation between the extension of white coat and reproductive performances of Holstein cows. Although coat color is heritable, it is unclear whether genetic selection based on color would benefit animal production agriculture.

Rectal temperature and respiration rates (or the combination) are commonly used to assess heat tolerance. Core body temperature results from all processes of thermoregulation, and rectal temperature is typically considered a good index of thermoregulatory capacity (Yousef, 1985). The heritability of rectal temperature is not well known, but appears to be medium to low (from 0.16 to 0.64; reviewed in Nardone, 1998). Genetic and phenotypic correlations between rectal temperature and productive (Johnson, 1987; Nardone *et al*., 1992; Spiers *et al*., 2004) and reproductive traits (Turner, 1982) have been reported.

Selection for heat tolerance within a breed may be an opportunity for improving animal performance in hot climates. Nardone and Valentini (2000) simulated selection schemes using a quantitative genetic approach for milk yield in adapted local breeds and heat tolerance (measured as rectal temperature) in cosmopolitan high-yielding dairy breeds. High-yielding breeds selected on the basis of rectal temperature showed more than double the annual progress of milk production than locally adapted breeds. Those authors concluded that selection based on rectal temperature in high-yielding breeds was the best (because heat tolerance can be improved in a few generations in high-yielding breeds),

whereas local breeds needed more than 30 generations to reach a comparable milk production.

Sire selection for heat tolerance may transmit important traits and should be considered. Carabano *et al.* (1990) did not find a sire by region interaction for milk and fat yield and concluded that bulls with daughters in one region would not be expected to be significantly re-ranked on records of daughters in another region of the United States. Zwald *et al.* (2003), exploring variables useful as an indicator of genotype by environment interaction, reported that bulls' daughters may perform differently in large *v.* small herds, or in herds with a hot climate *v.* herds with a cool climate. In a large study, Ravagnolo and Misztal (2000) reported that below a 72 THI, heritability for milk yield was 0.17 and additive variance for heat tolerance was 0. When the THI was above 72, the additive variance for heat tolerance was similar to a general effect, and the genetic correlation between the two effects was −0.36. Furthermore, Bohmanova *et al.* (2005) reported that bulls that transmit a high tolerance to heat stress have daughters with higher pregnancy rates, a longer productive life, but lower milk yields. Continued selection for milk yield without consideration of heat tolerance likely will result in greater susceptibility to heat stress. The selection for heat tolerance is possible, but the negative relationship of heat tolerance with milk yield needs to be recognized.

Hsp70 genotype and heat stress

The Hsp is a group of highly conserved proteins that are induced in both prokaryotes and eukaryotes by elevated temperatures or a variety of cellular stresses (Ross *et al.*, 2003). When heat shocked, the cells reduce their overall rates of gene transcription, RNA processing and translation, alter the activity of expressed proteins and, for a short period of time, increase expression of Hsp.

Hsp are traditionally classified by their molecular weight and the best understood are in the 110, 90, 70 and 60 kDa classes (Prohászka and Füst, 2004). These 'major' Hsp are constitutively expressed at 37°C in the absence of heat stress. The second group comprises 'minor' Hsp that are induced by glucose deprivation and include glucose-regulated proteins (grp) with molecular weights of 34, 47, 56, 75, 78, 94 and 174 kDa. A third group consists of low (about 20 kDa) molecular mass Hsp. As a protein, Hsp possess three intrinsic biochemical activities (Macario and Conway de Macario, 2007): (i) *Chaperone activity* that prevents the misaggregation of denatured proteins and assists in refolding of denatured proteins into their native conformations; (ii) Regulation of *cellular redox state*, which is best illustrated by Hsp32, better known as hemeoxygenase-1; (iii) Regulation of *protein turnover*, an example is ubiquitin, a protein expressed in unstressed cells, but upregulated by heat shock serving as a molecular tag marking proteins for degradation by proteasomes. The exact molecular targets for Hsp protection from cellular stress remain unresolved, but cell membranes, DNA and proteins have all been thought to be protected by Hsp.

Among Hsp, Hsp70 family (namely Hsp70.1 and Hsp70.2) has been most consistently associated with protection against conditions involving oxidative stress, such as ischemia, inflammation or aging (Favatier *et al.*, 1997). Furthermore, Hsp70 protein and its antibody have been identified as being involved in the pathogenesis of hypertension, atherosclerosis, coronary heart disease, acute heat-induced illness and heat stroke (Gromadzka *et al.*, 2001; Wu *et al.*, 2001; Pockley *et al.*, 2003; Jin *et al.*, 2004). Hsp70.1 and Hsp70.2 are polymorphic, potentially accounting for variation in their functions and susceptibility to stress tolerance (Ross *et al.*, 2003; Wu *et al.*, 2004; Zhou *et al.*, 2005). Single nucleotide polymorphisms (SNPs) in the coding region of *Hsp70* genes could affect peptide-binding kinetics or affinity of the Hsp70 proteins and ATPase activity, while nucleotide changes in the flanking regions (promoter and 5′, 3′-untranslated region (UTR)) might affect inducibility, degree of expression or stability of Hsp70 mRNA.

In addition, variation in *Hsp70* gene expression and polymorphisms has been positively correlated with variation in thermotolerance in *Drosophila melanogaster*, in *Caenorhabditis elegans*, in rodents and in humans (Hashmi *et al.*, 1997; Maloyan *et al.*, 1999; Sonna *et al.*, 2002; Gong and Golic, 2004; Singh *et al.*, 2006). In farm animals, some studies reported possible associations of SNP in the *Hsp70* genes with stress response and tolerance to heat. For example, studies in pigs and chickens examined polymorphisms in the promoter region, in the 3′-UTR and in the coding region of the *Hsp70* genes and these SNPs were associated with heat tolerance and stress response (Schwerin *et al.*, 2001 and 2002; Mazzi *et al.*, 2003). To date, few studies have reported SNPs in the bovine *Hsp70* genes. In beef cattle, 10 SNPs in the promoter region of the bovine *Hsp70.1* gene were identified and they were associated with weaning weights and pregnancy (Banks *et al.*, 2007; Starkey *et al.*, 2007). On the other hand, there are only two polymorphisms identified in the 3′ UTR of bovine *Hsp70.1* gene in dairy cattle (Grosz *et al.*, 1994; Adamowicz *et al.*, 2005), but the association between *Hsp70* gene polymorphism and production traits or thermotolerance was not studied. Cheng *et al.* (2009) reported a genetic polymorphism of *Hsp70.1* gene and its association with resistance to mastitis in Chinese Holstein.

As information concerning genetic polymorphism *Hsp* genes is sparse in dairy cattle, we searched for new SNPs in the bovine *Hsp70.1* gene in Italian Holstein cows under the SELMOL (molecular selection) project. The polymorphisms of *Hsp70.1* gene in 450 Italian Holstein dairy cows were studied for the association between polymorphisms, production traits and physiological responses to heat stress. We identified two new SNPs in the 5′UTR of bovine *Hsp70.1* gene in dairy cattle and distribution of genotypes and allele frequency are reported in Table 2.

The objective of SELMOL project is the genetic selection of heat stress-resistant genotypes without adversely affecting production. To accomplish this at the genomic level, the genes associated with acclimation, adaptation and thermotolerance need to be identified and studied. The central role that Hsp have in cytoprotection during heat stress is shown by the fact that Hsp overexpression protects against hyperthermia and cerebral ischemia during the heat stroke

Table 2 *Distribution of genotypes and alleles of the nucleotide sequence polymorphism within the 5′-UTR region of the bovine* Hsp70.1 *gene in Italian Holstein cows*

SNP	Number of animals			Allele frequency		Genotypes frequency		
5′UTR 895C/-	CC	C/0	0	C	0	CC	C/0	0
	298	128	20	81.2	18.8	66.8	28.8	4.4
5′UTR 1128G/T	TT	TG	GG	T	G	TT	TG	GG
	6	187	253	22.2	77.8	1.3	41.9	56.8

UTR = untranslated region.

(Lee *et al*., 2006). Heat shock (41°C) causes increased Hsp synthesis, decreased protein synthesis, mitochondrial swelling and movement of organelles away from the plasma membrane associated with cytoskeletal reorganization (Edwards and Hansen, 1996; Edwards *et al*., 1997; Rivera *et al*., 2003). As discussed above, Collier *et al*. (2008) reported the direct effects of thermal stress on cellular growth and ductal branching and on downregulation of genes associated with protein synthesis and cellular metabolism in BMECs. Maloyan and Horowitz (2002) reported that Hsp72 over-expression together with hormonal signals is an integral part of the heat acclimation repertoire.

Final remarks

Summer heat stress negatively impacts ruminant (especially dairy animals) performance in most areas of the world. The severity of heat stress issues will become more of a problem in the future as global warming progresses and genetic selection for milk yield continues.

Heat stress, both directly (mediated by hyperthermia) and indirectly (mediated by reduced nutrient intake and behavior changes), affects metabolic and physiological acclimation that may reduce the synthesis of useful products (milk and meat) and makes the animal more susceptible to illness. Improved knowledge of the functional relationship between animals and their environment, and of the physiological mechanisms of acclimation to environmental stresses may contribute to the adoption of procedures that improve the welfare and efficiency of production and reproduction. Accurately identifying heat-stressed ruminants and understanding the biological mechanism(s) by which thermal stress reduces milk synthesis, growth and reproductive indices are critical for developing novel approaches (i.e. genetic, managerial and nutritional) to maintain production or minimize the reduction during stressful summer months.

There are genetic differences within ruminants with respect to heat stress adaptations and these may provide clues or tools to select productive and thermotolerant animals. The role of Hsp in coordinating thermotolerance suggest that there is opportunity to study the association between polymorphisms within the *Hsp* genes, production traits and physiological response to heat stress in dairy cattle. These studies could support the development of breeding programs to improve animal performances during thermal stress.

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