ALLEVIATION OF HEAT STRESS WITH

TASCO IN DAIRY COWS

A Thesis presented to

the Faculty of the Graduate School

at the University of Missouri-Columbia

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

---

by

LUCIANA BARCHESI POMPEU

Dr. James E. Williams, Thesis Supervisor

JULY 2010

The undersigned, appointed by the Dean of the Graduate School, have examined the thesis entitled

ALLEVIATION OF HEAT STRESS WITH TASCO IN DAIRY COWS

presented by Luciana Barchesi Pompeu

a candidate for the degree of Master of Science

and hereby certify that in their opinion it is worthy of acceptance

_____

Dr. James E. Williams

_____

Dr. Donald E. Spiers

_____

Dr. Mark R. Ellersieck

ACKNOWLEDGEMENTS

First, I would like to thank Dr. Williams for giving me this opportunity of doing my Master's here at MU. Thank you for all the support, help, patience, answers, and advices. Also, I would like to thank Dr. Spiers for all the help, the over-hours meeting, all the answers, advices and support. I would never be able to understand the topic so well if it wasn't for you. Dr. Ellersieck, for all the handy-dandy little advices, making everything piece of cake!! Thank you Dr. Weaber, for the help in setting up the transmitters and in analyzing the core body temperature data. Thank you Hillary Vellios, Kevin Sargent and Nathan Feyerabend for helping me collect the data out at the farm! Thank you for all the rides (even with tornado warning!!), the availability in early mornings and hot afternoons!! Thank you for the people at the farm, John Denbigh, Eric Adkins, Kari Hoerath, for all the help with the "girls"! The study would not happen without all of you! Also, thank you all the faculty, staff and fellow grad students in the Department, who had part in my study and program.

Also, I would also like to thank my family. Daniel, for being my husband, my boyfriend, my "brother", my best-half! Thank you for everything you have always done for me! Mom, Carol and Bruno, for being always there for me in my entire life, and in the last 2 years, when, even far away, I could always count on you guys for everything!

Finally, I would like to thank all my friends who supported me during this program. George (Dr. Rottinghaus), for everything you have done for me, since the very first day I got in the US, in February 2007. Thank you for being my boss, my friend and my "American daddy"!! I will never forget everything you did for me, and I'll definitely miss you a lot… Thank you all my Brazilian friends, for being always there for me!

TABLE OF CONTENTS

iv

LIST OF TABLES

ix

LIST OF FIGURES

xii

xiii

ABSTRACT

The present study determined the impact of Tasco, *Ascophyllum nodosum*, on heat stress in dairy cows. Thirty-two Holstein cows were utilized in a randomized complete block design, during 63 days in summer 2008. Feed intake, milk production, respiration rate, body core and skin temperatures were measured for each cow. Ambient conditions in the free-stall barn were collected. The inclusion of 0.25% Tasco in the total mixed ration lowered dry matter intake and skin temperature for some days during the study, with no effect on milk yield. The same level of Tasco resulted in less of an increase in core and skin temperatures, as ambient temperature increased during the day. From the results, the inclusion of 0.25% Tasco may reduce heat strain of cows, maintaining lower body core and skin temperatures.

Typical effects of heat stress were observed in this study, including reduced feed intake and milk production, elevated respiration rate, skin and core body temperatures of dairy cows. Minimum vs. maximum or mean ambient temperature had more influence on the decline of dry matter intake and milk production. Cows that were more sensitive to heat stress were indentified within the group, and compared to the least sensitive cows. Animals differed in their response to heat stress, either by utilizing different mechanisms to cope or by not responding to it. By identifying the most sensitive cows, actions could be taken in order to alleviate the negative impact of heat stress in the herd.

CHAPTER 1

LITERATURE REVIEW

INTRODUCTION

Heat stress is a major concern for the US livestock industry, since it causes considerable losses every year, related to decrease in performance (production, efficiency and reproduction), health and well-being, and increase in mortality of animals. Many researchers try to assess the level of heat stress encountered by the animal, including measurements of environmental conditions, such as air temperature, relative humidity, air velocity, solar radiation, etc., and animals response, such as respiration rate, internal and superficial body temperature, panting, behavior, etc. The most widely used tool to assess heat stress is THI, which includes air temperature and humidity, but does not account for any animal response. Still, THI have been used to verify the possibility of the occurrence of heat stress, helping them to modify some management strategies in order to alleviate the effects of hot weather. These alterations may include the use of fans, sprinklers, shade, as well as a shift in the feeding strategies and modifications of the ration. Moreover, the use of feed additives may have an impact on the alleviation of heat stress. Yeast culture, supplemental fat, and seaweed have been evaluated for their effects in alleviating heat strain in cattle. Although no feed additive has definitively been found to be effective in reducing heat strain, a brown seaweed (*Ascophyllum nodosum*) was shown

to reduced body temperature (Archer et al., 2007; Spiers et al., 2004a) and improve immune function during heat stress periods, with no negative effect on performance (Allen et al., 2001a; Fike et al., 2001; Saker et al., 2001). Thus, the goal of this literature review is to present the general effects of heat stress in dairy cattle and some means to alleviate this stress and improve health and performance of animals. The inclusion of brown seaweed to the diet will be discussed as one way to reduce heat strain of cows, and possibly improve production.

HEAT STRESS

Heat strain is a negative balance between the heat produced by the animal and the amount of heat the animal can dissipate to the ambient. It occurs when the ambient conditions are above the upper threshold of the zone of thermal comfort, which varies among species and physiology of individual animals (Armstrong, 1994; St-Pierre et al., 2003). Within a zone of thermal comfort, animals expend less energy above maintenance to cool or heat the body. However, when ambient temperature is above or below this zone, animals need to adjust their metabolism in order to maintain homeostasis (Kadzere et al., 2002). When environmental conditions surpass a threshold, performance and health of animals are affected, so animals may utilize behavioral, physiological and immunological means to cope with the adverse conditions (Hahn, 1999).

Lactating dairy cattle are one of the most affected animals because of the large amount of metabolic heat produced during lactation. The thermoneutral zone for lactating cows ranges from 5 to 25°C, depending on breed, size and level of production, and above this upper limit, cows must expend extra energy to dissipate heat and maintain normal

body temperature (Roenfeldt, 1998). Below a threshold of ambient temperature, milk production is constant (Ravagnolo et al., 2000); however, as temperature rises above 26°C, cows may start experiencing heat strain, such as increase in body temperature and respiration rate, decrease in feed intake and milk production, change in milk composition, and limitation of reproductive performance (Berman et al., 1985; Kadzere et al., 2002).

Genetic selection for higher milk production causes dairy cattle to become more vulnerable to heat stress, since the higher milk yield is accompanied by a higher feed intake and elevated metabolic heat production (Kadzere et al., 2002). Moreover, the body weight of cows has became greater, since larger cows produce more milk (Kadzere et al., 2002). Berman (2005) estimated that an increase in milk production from 35 to 45 kg/day could reduce the upper critical temperature by 5°C. A study performed in Turkey demonstrated that high-producing cows (> 25 kg of milk per day) had a 16.1% decrease in milk production during summer, while low-producing had a reduction of only 11.6% (Tapkı and Şahin, 2006). Moreover, during a heat wave in 1977, high-producing cows were the most affected by heat stress, accounting for 71.5% of the deaths, while only 9.4% of the cows that died were low-producing cows (Oliver et al., 1979). Heifers are less affected by heat stress because they produce less metabolic heat and have greater surface area relative to internal body mass, contributing to more efficient heat dissipation (West, 2003). Moreover, the effects of heat stress are influenced by the stage of lactation; early lactation cows are more susceptible to heat stress, and less able to cope than late lactation or dry cows (Araki et al., 1984; Igono and Johnson, 1990). In addition to the higher level of feed intake and milk produced, during early lactation cows are in negative

energy balance, and so, need to mobilize body reserves, increasing metabolic heat production (Kadzere et al., 2002).

Management and feeding strategies, as well as genetic parameters, such as animal size, breed, stage of lactation, and level of production, have an influence on the tolerance to heat stress (Linvill and Pardue, 1992; St-Pierre et al., 2003). However, the estimation of heat stress effects in cattle is complicated because of their ability to acclimate to environmental modifications, which can take from days to weeks to be completed (Collier and Zimbelman, 2007). Finally, the susceptibility to heat stress and capacity to adapt to the conditions are variable within a population of animals, causing some individuals to have a greater response to heat stress than others; this larger response may be related to the genetic diversity, health and nutritional status (Hahn, 1999). According to Nardone et al. (2010), the selection of animals based on production traits will soon be replaced by a selection based mainly on adaptability to heat stress.

Economic impact of heat stress

Heat stress is one of the main causes of economic losses in livestock production in the United States. St-Pierre et al. (2003) estimated that these losses were $ 2.4 billion dollars per year, with the dairy industry contributing $ 1.5 billion dollars. With the utilization of heat abatement systems, the total loss was reduced to $ 1.7 billion dollars per year, and the loss in the dairy industry reduced to $ 0.9 billion dollars (St-Pierre et al., 2003). It was estimated that across the United States the decline in milk production related to heat stress ranged from 68 kg/cow/year in Wyoming to 2,072 kg/cow/year in Louisiana; Missouri was ranked as the tenth state in milk loss, with an average of 936 kg/cow/year reduction in milk production, which could be considered as a consequence

4

of a 464 kg/cow/year decrease in DM intake. In reproduction, there is an increase in average days open, ranging from 4.3 in Wyoming, 29.0 in Missouri, to 59.2 in Florida, and in annual cull rate per 1000 cows, ranging from 2.7 in Wyoming, 31.5 in Missouri, to 88.2 in Louisiana (St-Pierre et al., 2003). In this study, heat stress conditions were assessed by calculating a temperature-humidity index (THI) load, corresponding to the sum of time THI was above a certain threshold, defined for each class of animal. For dairy cattle, the THI threshold was considered to be 70, a little lower than the standard THI of 72 widely used; the authors justified the use of 70 for THI because the currently higher milk production might cause cows to be more susceptible to heat stress. Wyoming presented the lowest THI load per hour (4.0 units/h) while Texas had the greatest value (8.0 units/h); Missouri presented 7.3 units/h. Florida presented 48.6% of all annual hours subject to heat stress conditions, which was the highest frequency across all states; Missouri presented 21.4% of the annual hours with heat stress. Reduction in losses from the use of heat abatement systems was also calculated. The authors found fans and sprinklers reduced losses from 1,458 to 848 million dollars per year across all states. When the heat abatement system was utilized, the annual total loss, considering production, reproduction, and mortality was greatest for Texas (130 million dollars/year), California (118 million dollars/year) and Wisconsin (57 million dollars/year); Missouri had 29 million dollars of losses per year. This corresponded to about 74% of the total loss without any heat abatement utilization (St-Pierre et al., 2003).

Linvill and Pardue (1992) reported the negative effects of hot weather on dairy cows were more evident with a sudden rise in air temperature, during early summer or a warm front during other seasons. Heat waves and extended periods of high THI, without

5

sufficient nighttime cooling, may cause serious heat strain and death of animals (Brown-Brandl et al., 2005a; Oliver et al., 1979). In 1977, a heat wave was reported to cause the death of 725 dairy cows in California, and also a huge decline in milk production, accounting for one million dollars of losses (Oliver et al., 1979). In 2006, a more recent heat wave in California caused losses over one billion dollars for the dairy industry, corresponding to animal deaths and reduced milk production (Collier and Zimbelman, 2007). Other heat waves in Iowa and in Nebraska were found to cause deaths of large numbers of cattle, contributing to huge economic losses (Brown-Brandl et al., 2005a).

Mechanisms for heat loss

Heat gain from solar radiation and metabolism occurs if mechanisms for heat loss are not sufficient to dissipate the heat; lower temperatures at night favor heat flow from the body to the environment, dissipating the stored heat (Finch, 1986). As ambient temperature increases, heat loss is shifted from non-evaporative (convection, conduction, and radiation) to evaporative mechanisms (sweating and panting) (Kadzere et al., 2002). Increase in evaporative water loss from the skin is the first attempt to cope during initial phase of heat stress, followed by an increase in respiratory heat loss. Elevation of core body temperature will occur if skin and respiratory losses are not enough to counteract input of heat, and excess heat is accumulated (Berman, 2005). Moreover, an increase in body temperature may happen if ambient conditions are unfavorable for evaporative heat dissipation, such as during heat stress and elevated humidity, increasing body heat load (West, 2003). Besides the changes in the heat loss mechanisms, animals also decrease their feed intake, as another attempt to reduce metabolic heat production (Hahn, 1999).

Under heat stress, about 15% of the endogenous heat is lost directly from the body core through respiration, and the rest is transferred to the skin and lost through non-evaporative and evaporative mechanisms (Finch, 1986). Skin heat loss depends on the temperature gradient between skin, air and solid objects in the environment (Kadzere et al., 2002). According to Berman et al. (1985), peripheral vasodilation is not a major factor contributing to heat loss in cattle, because of their small surface area to mass ratio. Moreover, the increase in blood flow to the periphery as a cooling mechanism may cause a change in nutrient metabolism decreasing milk production (West, 2003).

Effects of heat stress in dairy cattle

Dairy cow's main responses to heat stress include reduced feed intake and appetite, increased water intake, decreased urinary and fecal water loss, reduced activity and seeking for shade (Kadzere et al., 2002; West, 2003). Respiration rate, evaporative water loss, body temperature, blood flow to periphery and sweating rate are increased, depending on the level of heat stress (Armstrong, 1994). Moreover, there is a change in metabolic rate, maintenance requirements, and blood hormone production and release (Armstrong, 1994) all negatively impacting physiology and production. Heat stress affects blood acid-base chemistry of cows, by lowering blood CO_2 and HCO_3 concentrations, and elevating blood pH, possibly causing respiratory alkalosis (Schneider et al., 1988). Also, an increase in urine pH may occur, because of increased excretion of HCO_3 by the kidney, in order to maintain an adequate $HCO_3:CO_2$ ratio to the buffering system (Schneider et al., 1988). Moreover, potassium excretion by sweating and sodium excretion in the urine are increased during hot weather (Collier et al., 2006; Kadzere et al., 2002).

During heat stress, nutrient utilization and rumen function are altered, possibly attributed to a shift in blood flow from the digestive system to peripheral tissues and respiratory system (Bernabucci et al., 2009; Finch, 1986). Rumen contractions and motility are decreased during hot weather, reducing rumen activity (Collier et al., 1981) and increasing mean retention time (Schneider et al., 1988). Also, liquid and solid dilution rates are decreased during heat stress, and this reduction may not be dependent on level of feed or water intake (Bernabucci et al., 1999; Schneider et al., 1988).

Rumen microorganisms and production of volatile fatty-acids (VFA) are also affected when cows are exposed to hot conditions. Schneider et al. (1988) showed a reduction of total VFA concentration for heat-stressed cows, attributed to a lower feed intake or higher dilution, because of the higher intake of water. The concentration of butyric acid was slightly increased. In contrast, Kelley et al. (1967) found a great reduction in acetate and propionate concentrations during heat stress, accounting for the majority of the decline in VFA levels; they also found a small decrease in the concentration of butyrate. Moreover, the authors found an increase in the acetate: propionate ratio, from 2.8:1 to 4.4:1 during heat stress. Under heat stress conditions, cows reduce intake of forage relative to concentrate, if they have the opportunity to select, which reduces heat production from rumen fermentation (Bernabucci et al., 1999). Diet digestibility was improved when dairy cows were exposed to short-term heat stress, and it was reversed under extended exposure (Bernabucci et al., 1999). In sheep, short-term heat stress did not affect DM intake and digestibility of DM, OM, NDF and ADF; however, a decrease in passage rate and increase in retention time were observed (Bernabucci et al., 2009). On the other hand, ewes subjected to long-term exposure to

heat had decreased diet digestibility, rumen pH and osmolarity, reduced cellulolytic and amylolytic bacteria concentration and slower passage rate (Bernabucci et al., 2009). The study suggested that digestibility depends on extension of exposure to heat stress. Also, after return to thermoneutrality, digestibility and passage rate did not return to normal levels for the next 15 days, suggesting that rumen function may be temporarily impaired (Bernabucci et al., 2009).

Heat stress also alters the eating pattern of cattle. Dairy cows at thermoneutrality eat larger meals, soon after feed is offered, while heat-stressed cows wait until air temperature is cooler to eat, which generally occurs between 2100 and 0900 hours (Schneider et al., 1988). Brown-Brandl et al. (2005a) found that beef steers exposed to heat stress, using simulated cyclic temperature and heat waves, modified their eating behavior with reduced number of meals and rate of eating, while maintaining similar duration of eating. The smaller amount of feed consumed is a way to cope with heat stress. Initial exposure to heat stress in climatic chambers changed the feeding behavior of *Bos taurus* steers, with a shift to more frequent eating of smaller meals; however, after animals were acclimated, the pattern of feeding before heat exposure was reestablished with less frequent and larger meals (Hahn et al., 1990).

Heat stress is also known to cause deficiency in the defense mechanism, which increases the animal's susceptibility to infectious disease (Kelley, 1980). Olde Riekerink et al. (2007) found higher somatic cell count in cows during summer months of May and August, and higher bulk milk somatic cell count in August and September. Moreover, incidence of clinical mastitis by two pathogens, *Streptococcus uberis* and *Escherichia coli*, was higher in summer months. In contrast, Collier et al. (1981) did not find

differences in milk somatic cell count between cows with or without access to shade, representing different levels of heat stress; cows with no-shade had numerically higher incidence of mastitis. Finally, parasites and vectors of diseases are more prevalent during the hot and humidity season, impacting health of animals (Kadzere et al., 2002).

Tools for assessing heat stress

In order to assess the levels of heat stress to which animals are exposed to and identify its effects on thermal status and performance, certain indices may be utilized. Temperature-humidity index (THI) combines air temperature with relative humidity in order to represent the level of heat load to which animals are exposed. The combination of air temperature and relative humidity as a tool to verify heat stress conditions is especially important for places where the humidity is usually elevated (Bohmanova et al., 2007). The high moisture of the air impedes efficient evaporative heat loss by sweating and panting, and also compromises the efficiency of evaporative cooling systems in lowering animal's body temperature (Bohmanova et al., 2007). However, some authors indicated that THI may underestimate heat stress for modern dairy cattle, because milk yield has increased significantly since the development of the index. Additionally, it may not accurately represent heat load for animals, because it does not account for other important ambient conditions, such as solar radiation and wind speed (Berman, 2005; Mader et al., 2006). Authors found that daily mean vaginal temperature of lactating dairy cows housed in pens without shade was correlated to daily mean dry bulb temperature, black globe temperature and RH, but not with daily mean THI (Araki et al., 1984).

Milk losses due to heat stress were compared in two different locations in the United States one with a hot and dry summer, and the other with a hot and humid summer

10

(Bohmanova et al., 2007). The authors found that heat stress occurs at lower levels of ambient temperature when the humidity is elevated, while in drier climates, cows exposed to higher ambient temperature may have the same level of heat strain. Moreover, the authors suggested that THI with higher weighting for humidity is more appropriate to be utilized in humid climates, while THI with higher weighting for ambient temperature should be utilized in drier climates. However, other authors (Dikmen and Hansen, 2009) indicated that the use of ambient temperature alone to determine heat stress is virtually as good as THI, since a high correlation existed between dry bulb temperature and 7 different THI definitions tested.

Ravagnolo et al. (2000) suggested that the use of ambient conditions alone is not enough to predict the depression in milk production during heat stress periods. The relationship between milk yield and THI, calculated by using daily maximum air temperature and daily minimum RH, resulted in a very small coefficient of determination ($R^2 = 0.005$), suggesting that ambient conditions explain only a small portion of the decline in milk production during hot weather (Ravagnolo et al., 2000). One reason the authors gave to the low correlation was the lack of lag incorporation to the model, thus, not truly expressing the relationship between milk production and ambient conditions. Also, they utilized data over a whole year, incorporating heat stress and non-heat stress periods all together, which may reduce tremendously the correlation coefficient, since at cooler periods, milk production is not correlated to ambient temperature (Berman et al., 1985; Ravagnolo et al., 2000).

In order to assess heat strain of cows, the use of animal's thermal responses may be more accurate than the use of ambient conditions. According to Collier and

Zimbelman (2007), skin surface temperature can be a useful tool for assessing the level of heat stress, since it represents the microenvironment around the animal, accurately representing the thermal impact of the environment on the animal. Skin temperature is the first physiological response to change with the external environment (Spiers et al., 2004b); moreover, skin temperature is fairly easy to measure with infrared guns. However, the use of skin temperature as an indicator of stress comes with some problems. Because of the large variation across different areas in the animal's body, it is hard to identify one site to accurately express the influential skin temperature (Spiers et al., 2004b). Moreover, the entire extent of an animal's skin is not simultaneously affected by ambient factors, like wind and solar radiation, thus, contributing to greater variation among individual sites.

Another approach to assess heat stress could be utilizing respiration rate as the strain indicator. Under heat stress, respiration rate, which is a thermoregulatory effector, is affected first, before a change in body temperature occurs (Spiers et al., 2004b). Studies with feedlot cattle indicated respiration rate is a good indicator of heat stress, accurately representing animal and environment conditions; also, it is easy to monitor and do not require costly equipment (Brown-Brandl et al., 2005b; Gaughan et al., 2000). Moreover, it has been shown to parallel changes in feed intake, representing a sensitive indicator of changes in production (Williams et al., 2009). Respiration rate may decrease during hot periods due to a change in the respiration rate dynamics from rapid open mouth panting to a slower deep phase open mouth panting; this could be an indication of animals failing to cope with heat stress (Gaughan et al., 2000).

Furthermore, internal body temperature can be considered a good indicator of heat stress in cattle, since it is kept almost constant at thermoneutral conditions (Kadzere et al., 2002). Hahn et al. (1990) compared 4 locations in the body to measure temperature of steers housed in climatic chambers and exposed to 50 hours of cycling ambient temperature. They found that tympanic and rectal temperatures are better predictors of body temperature than sub-dermal upper shoulder and sub-dermal upper flank, and, between the internal locations, tympanic expressed thermoregulation more accurately than rectal temperature (Hahn et al., 1990). When comparing rectal temperature, respiration rate and indices combining the two parameters and ambient conditions, rectal temperature was the better indicator of heat strain in cows, as observed by a decline in feed intake and milk production (Spiers et al., 2004b). This suggests that a change in internal body temperature has to occur before any change in performance is observed.

Finally, some authors suggest that a good indicator of heat stress should include weather parameters, such as air temperature, relative humidity, wind speed and solar radiation, as well as an animal's characteristics, such as sex, coat color, health status, body size, etc. (Brown-Brandl et al., 2005b). The environmental conditions combined with animal's characteristics is considered as the input in the system, and the response of the animal (i.e. core body temperature, respiration rate, skin temperature, sweating rate, panting rate) is viewed as the output (Berman, 2005). Even though the inclusion of many variables may increase the precision of an index, it may cause the predictor to be complex and hard to interpret, especially by producers. An easy, fast and reliable indicator of heat stress would be a useful tool for producers, so, they could determine the onset of a heat stress event and take preventive and corrective actions.

Physiological responses to heat stress

   Thermal status and the environment. There is extensive documentation that ambient conditions affect thermal status of different species of animals. For dairy cows, rectal temperature is not dependent on ambient temperature below 24ºC, but it is related to energy metabolism; as ambient temperature increases above 25ºC, rectal temperature starts rising (Berman et al., 1985). For respiration rate, the threshold of ambient temperature is $21.3^{o}C$, which is considerably lower than the threshold for rectal temperature (Hahn, 1999). For beef cattle housed in environmental chambers and subjected to cyclical ambient temperature, an increase of 4.3 breaths/min for every degree increase in ambient temperature was found (Hahn, 1999). In addition, respiration rate rises above 50 to 60 breaths/minute as ambient temperature increase above 25ºC (Berman et al., 1985).

   There is some speculation that the ambient conditions at night are more important in affecting heat strain of cows than those conditions during the day. If the ambient temperature at night does not fall sufficiently to allow animals to cool down, there will be an accumulation of heat in the body, contributing to the increase in body temperature during the day (Brown-Brandl et al., 2005a; Gaughan et al., 2000; Gaughan et al., 2007). Gaughan et al. (2000) compared daytime with nighttime cooling and found that the nighttime cooling was more efficient in lowering body temperature of steers housed in environmental chambers; nighttime cooling reduced overall respiration rate and rectal temperature, even though the maximum values for those parameters were higher than that of steers cooled during the day. The authors suggested that animals cooled at night were exposed to a longer period of low temperatures, favoring animals to cope with heat stress.

Animals may store heat during the day, when conditions are not favorable for heat loss, and dissipate it at night when ambient temperature is lower by increasing respiration rate (Gaughan et al., 2000).

Ambient conditions may not influence the animal's thermal status immediately, and the delay varies depending on the ambient conditions and the animal parameter being affected. Also, the delay in the response is influenced by body size and the ability of the animal in regulate production functions, heat production, and heat loss (Igono and Johnson, 1990). Dairy cows in an open housing situation, with free-stalls and access to shade, had peak rectal temperature lagging behind peak black globe temperature by 5 hours, and this delay was reduced to 2 hours when sprinklers were utilized (Igono and Johnson, 1990). For *Bos taurus* cattle housed in environmentally controlled chambers, tympanic temperature lagged behind air temperature by about 2 to 5 hours (Hahn et al., 1990). Core body temperature measured by a transmitter implanted in the abdominal cavity lagged behind ambient conditions by 1 to 5 hours (Brown-Brandl et al., 2005b).

When evaluating the respiration rate response of steers housed in environmental chambers, Gaughan et al. (2000) found that respiration rate lagged behind an increase in ambient temperature by 0 to 3 hours with an average of a 2 hour delay, and tympanic temperature lagged behind respiration rate by 2 hours. This finding was supported by other authors who evaluated beef cattle subjected to cyclical ambient temperature in chambers; they found a lag of 2 hours between ambient temperature and respiration rate (Hahn, 1999). In contrast, when beef steers were housed in pens and exposed to solar radiation and wind, respiration rate lagged behind solar radiation by about 1 hour (from

15

0.5 to 1.25 hours), suggesting a different response whether the animal is housed inside or outside (Brown-Brandl et al., 2005b).

The delay in response of an animal's thermal status to changes in the ambient conditions may delay the observation of stress by the producer, delaying possible actions to minimize heat stress effects. It has been demonstrated that animals respond first with respiration rate, and later with changes in core body temperature (Spiers et al., 2004b). Respiration rate of beef steers housed in climatic chambers started to increase at lower ambient temperatures, before rectal temperature increased, indicating that respiration rate is an initial mechanism of heat loss to prevent an increase in core body temperature (Williams et al., 2009). Thus, respiration rate might be a better indicator of environmental stress, which can be identified earlier and, as long as corrective actions are taken, avoid an increase in core body temperature and the detrimental effects of heat stress on cows.

Performance and the environment. During heat stress periods, animals reduce their feed intake, in order to reduce the heat produced by digestion, which is especially important for ruminants (Kadzere et al., 2002). This reduction in intake is one of the causes for the depression in milk production. Cows housed in climatic chambers, and exposed to three weeks of cyclic heat stress conditions, had a 33% decline in DM intake in the first two weeks of heat stress, but the intake increased by 15% during the third week, suggesting that cows were acclimating to the hot environment (Shwartz et al., 2009). Moreover, milk production was reduced from the first to the last week of heat stress, being 33% lower when compared to thermoneutral conditions in the third week of the study. Because milk production was not increased with the increase in intake during week 3, the authors suggested that other factors, besides feed intake, may dictate milk

16

production during heat stress periods, and indicated that post-absorptive metabolism may influence milk synthesis (Shwartz et al., 2009). This was supported by other studies, which demonstrated that only 35% of the decline in milk production during heat stress was attributed to lower feed intake and that heat stress had a direct effect on physiological responses that would modify endocrine status, maintenance requirements, post-absorptive metabolism and nutrient partitioning, contributing to the reduced milk production (Rhoads et al., 2010; Rhoads et al., 2009).

Ravagnolo et al. (2000) suggested the drop in milk production on a specific day is related to the maximum, minimum or average ambient temperature and relative humidity of the preceding 24-hour period, and to the length of previous and current heat stress periods. Collier et al. (1981) found a greater response in milk yield to black globe temperature with 24 to 48 hours of delay. In contrast, Linvill and Pardue (1992) found that the decline in milk production was well correlated with the number of hours during the past 4 days, when THI was above 74 and 80. Evaluating the effects of heat stress in Jersey cows, Holter et al. (1996) found the minimum daily THI was better correlated to feed intake and 4% energy-corrected milk than the maximum THI. Moreover, they found feed intake starts to decrease when minimum THI was above 56 to 57, which corresponded to a maximum THI of about 71.

Production and weather data from dairy farms in Georgia, combined over 7 years, suggest milk production starts to decrease when ambient temperature rises above $24^{o}C$ (Ravagnolo et al., 2000). Moreover, each unit increase in THI, when calculated by using daily maximum air temperature and daily minimum RH, above 72 causes a 0.2 kg decrease in milk production, with a concomitant depression in fat and protein yield

17

(Ravagnolo et al., 2000). In contrast, Zimbelman et al. (2006) reported a decrease of about 2.2 kg/day in milk production of high-producing dairy cows, when minimum THI rose above 65 or when average THI was equal to 68 for more than 17 hours. The authors suggested these two values of THI might serve as thresholds for cooling the cows, replacing the standard THI threshold of 72.

   Performance and thermal status. It is believed that the ambient conditions indirectly impact production traits, by affecting the animal's thermal status, which in turns influences performance (Johnson, 1965). Thus, a depression in performance is probably caused by an increase in animal's core body temperature. According to Igono and Johnson (1990), when rectal temperature is above 39ºC or air temperature is higher than 27ºC, milk production and fertility are negatively affected. Moreover, milk production of high-producing cows decreases when rectal temperature is greater than 39ºC for more than 16 hours (Igono and Johnson, 1990). Maust et al. (1972) revealed that elevated rectal temperature reduced feed intake of lactating dairy cows; additionally, rectal temperature affected milk yield 3 days later. This suggests core body temperature affects intake more readily than milk production. It is possible that the decrease in milk production 3 days later was an indirect consequence of the elevated core body temperature, and a direct effect of the reduced feed intake, reducing energy available for production functions. Spiers et al. (2004b) found rectal temperature is a better predictor of the decline in feed intake and milk production than respiration rate and indices of heat strain combining rectal temperature and respiration rate, and both combined with skin temperature or THI. These findings suggest a change in body temperature is necessary to produce a change in feed intake and milk production (Spiers et al., 2004b). In contrast,

Dikmen and Hansen (2009) did not find a significant relationship between milk yield and rectal temperature, and they suggested some cows may have a superior thermoregulatory capacity, so they are able to produce more milk.

Furthermore, the level of production influences an animal's body temperature. High-producing cows have higher metabolic rate, and also higher intake, in order to supply enough nutrient and energy needs for milk production, increasing the heat load in the body (Kadzere et al., 2002). Research has shown daily milk yield has an effect on rectal temperature of high-producing cows (> 24 kg/day fat-corrected milk), but it is not affected in low-producing (< 24 kg/day fat-corrected milk) (Berman et al., 1985).

Partial acclimation of beef cattle to heat stress has been reported to occur after 3 to 4 days of exposure to hot conditions, when core body temperature starts to decrease and feed intake increases toward thermoneutral level (Hahn et al., 1990). The rate of acclimation was verified to be around 0.1 to 0.4°C decrease in tympanic temperature per day (Hahn et al., 1990). Moreover, physiological adjustments to heat stress in this study appeared to be complete after 8 days. Another study analyzing heat strain in beef steers housed in a climatic chamber and exposed to 23 days of heat stress revealed that complete recovery of feed intake was obtained only after 10 days at thermoneutrality (Williams et al., 2009). In the same study, after reestablishment of thermoneutrality, respiration rates returned to normal level, while rectal temperature undershot, possibly indicating adaptation to heat stress (Williams et al., 2009).

Means to alleviate heat stress in dairy cows

  Environmental strategies. In order to alleviate the effects of heat stress for dairy cows, some approaches are proven to be effective, and they include modifications of the animal's environment, management practices and feeding strategies.

  Environmental modifications are usually related to shade, ventilation and cooling, which protects animals from direct and indirect solar radiation and improves cooling by convection, conduction, radiation and evaporation (Jones and Stallings, 1999; West, 2003). The benefits of shade to lactating dairy cows were reported in New Zealand; cows in pasture with access to artificial shade had reduced mean vaginal temperature and higher milk yield compared to cows without access to shade (Kendall et al., 2006). Muller et al. (1994a) reported a higher respiration rate and rectal temperature at 1100, 1300 and 1500 h of cows without access to shade, during hot days; however, in days when the maximum ambient temperature was below 25ºC, shade did not have any effect on thermal status of cows. Lactating dairy cows with access to shade had higher feed intake and milk yield, spent more time eating during the day and lying down than cows with no access to shade, which had higher water intake and spent more time standing (Muller et al., 1994b, c). By standing, cows increase their exposed surface area to the environment, contributing to increased heat dissipation (Kendall et al., 2006). Igono and Johnson (1990) found that the use of shade and sprinklers for dairy cows in open housing conditions with free-stalls was more effective in reducing heat stress, compared to the use of shade alone. Cows in shade and sprinklers had lower mean rectal temperature in the morning and afternoon, lower duration of rectal temperature above 39ºC, and higher milk production, compared to only shade (Igono and Johnson, 1990). Moreover, the authors

showed by utilizing shade with sprinklers and fans, rectal temperature of cows was further decreased, and the negative impact of heat stress was minimized (Igono and Johnson, 1990). Dairy cows had a lower response to high ambient temperatures when forced-ventilation was applied in the barns, observed both by a reduced rate of increase in rectal temperature, relative to the increase in air temperature, and by a lower mean value for rectal temperature and respiration rate (Berman et al., 1985). The authors suggested that forced-ventilation may be efficient in reducing heat strain of cows when ambient temperatures are above 25ºC and up to 36ºC, when approaching core body temperature.

Nutritional strategies. During heat stress, nutrient intake is reduced and energy for maintenance is increased (Fox and Tylutki, 1998), causing cows to be in a negative energy balance, and limiting milk synthesis (Moore et al., 2005). Thus, in order to supply enough nutrients for the animal and avoid the decline in milk production, the diet should be modified to account for lower DM intake, dietary heat increment, greater nutrient requirement, and minimal excess of nutrients (West, 2003). Diets should contain a lower amount of forage, since high forage diets may increase heat production, and a higher amount of concentrate to stimulate intake and increase energy density of the diet (Staples, 2007). The energy density can also be increased with supplemental fat (Drackley et al., 2003); however, either the higher content of grain or the addition of fat needs to be done with caution in order to avoid problems associated with acidosis (Kadzere et al., 2002) and decreased fiber digestion (Drackley et al., 2003). Lactating dairy cows fed high-grain or high-fat diets during the summer had increased milk production but reduced milk fat compared to a typical diet; milk protein was increased with the high-grain diet and decreased with the high-fat diet (Drackley et al., 2003). However, cows on the high-grain

diet had higher morning rectal temperature and higher respiration rate compared to cows on the high-fat diet, suggesting that this modification of the diet may increase the level of heat strain in cows. Restrictive feeding has also been demonstrated to reduce core body temperature in beef steers, either by reducing metabolic rate or metabolic heat load (Mader et al., 2002).

Protein content of diets should be increased during heat stress to account for the lower intake, but excess should be avoided, since it reduces ME and the excretion of excess nitrogen increases heat increment; RUP may be increased in the diet, but RDP should not exceed 61% of dietary CP (Huber et al., 1994; West, 2003). Also, diets with high dietary cation-anion differences can balance the higher mineral excretion during hot weather, avoiding negative effects of heat stress and increasing DM intake and milk production (West et al., 1991).

The inclusion of yeast culture and fibrolytic enzymes (cellulases and hemicellulases) may also be beneficial for heat-stressed cows (Shwartz et al., 2009). Supplementation of diets for dairy cattle with yeast culture improves feed efficiency (fat-corrected milk per DM intake) (Moallem et al., 2009; Schingoethe et al., 2004), and increases intake, milk production, and fat yield during heat stress periods (Moallem et al., 2009). In contrast, other studies show the inclusion of yeast culture does not affect feed efficiency, and reduces DM intake and milk production of heat-stressed cows; however, these differences disappear when the parameters are evaluated relative to percent of body weight (Shwartz et al., 2009). In this same study, it was found the yeast culture diet occasionally reduced rectal, tail head, and shoulder temperatures, but had no effect on rump temperature, respiration rate, and sweating rate (Shwartz et al., 2009).

Finally, water is one of the most important factors to combat heat strain in cows. Water should be available at all times and at small distances from animals, since the location of the water troughs influence the intake (Staples, 2007). Cows drink higher amounts of water during heat stress, which contributes to body water content and increases evaporative cooling by sweating and respiration (Staples, 2007). Water is closely related to DM intake and milk production of dairy cows (West, 2003). The temperature of the water also influences the amount of water consumed (Staples, 2007). Cows consuming chilled water (10.6ºC) had higher DM intake and milk yield, and lower respiration rate and rectal temperature compared to cows offered water at 27ºC (Wilks et al., 1990). However, when the preference of cows for chilled or warm water was tested, it was found that cows prefer warm over cool water 97% of the time (Wilks et al., 1990).

SEAWEED ASCOPHYLLUM NODOSUM

Ascophyllum nodosum is a brown seaweed harvested from the North Atlantic Coast of Canada. The main uses of the seaweed products are in industrial processes and products, as a fertilizer of soils and plants, and as a feed additive; in human food, the seaweed is utilized as a constituent of health food tablets and as a viscosity control agent ingredient (alginates) for sauces, instant mixes and pie filling (Sharp, 2005). The composition of the seaweed varies with season, immersion/submersion period, diurnal variation, part of the plant, and location (Sharp, 2005). In general, it is a good source of minerals, vitamins (B, C, E) (Burtin, 2003), plant growth regulators, such as auxin, gibberellins, cytokinin (Zhang, 1997), and polysaccharides (Burtin, 2003).

23

One of the main benefits of brown seaweed is the antioxidant activity. In the body, there is a balance between the production of oxygen free radicals and antioxidants, which play a role in removing free radicals. When an imbalance occurs, the amount of free radical increases, resulting in oxidative stress. A mild oxidative stress will cause an increase in the production of antioxidants, reestablishing the equilibrium; however, under major stress, the balance is not regained, and cell death may occur (Allen et al., 2001b). Some compounds with antioxidant activity include vitamin E, selenium, ascorbic acid and the enzyme superoxide dismutase. Brown seaweeds are rich in vitamin E (α-, β- and γ-tocopherol), containing higher amounts of this vitamin than other types of algae (Jensen, 1969). In addition, *A. nodosum* is rich in polyphenols and carotenoids, which may also have antioxidant activity (Burtin, 2003).

Cell wall structural polysaccharides are largely found in *A. nodosum*; alginate is mainly used by the hydrocolloid industry, while fucoidans have important therapeutic functions, such as anti-thrombotic, -coagulant, -inflammatory, -cancer, and -viral (Burtin, 2003). Moreover, seaweeds are rich in minerals, macro-elements and trace-elements. The concentration of iodine and calcium are elevated; the absorption of calcium may be limited because it is strongly associated with polysaccharides, while iodine association with the polysaccharide is weak, and thus, absorption is high (Burtin, 2003). Brown seaweeds are not high in protein content, and the presence of phenols may limit protein availability (Burtin, 2003). Also, brown seaweeds are high in polyunsaturated fatty-acids ω-3 (eicosapentanoic acid) and ω-6 (arachidonic acid), which are known to prevent cardio-vascular diseases (Burtin, 2003). *Ascophyllum nodosum* also contains some sterols, such as fucosterol, which may prevent high cholesterol in blood (Knights, 1970).

24

Tasco

Tasco (Acadian Seaplants Limited; Nova Scotia, Canada) is a proprietary product

obtained from *Ascophyllum nodosum*, and commercialized as Tasco-Forage, a water

soluble extract produced by alkaline hydrolysis and utilized in plants and soils; Tasco-

EX, an extract also obtained from alkaline hydrolysis of the seaweed, or Tasco-14, an

intact form of *A. nodosum*, obtained through solar drying (Allen et al., 2001b). These two

products have been used as feed additives in livestock feed. Tasco is recognized as safe

(GRAS)-approved feed additive, with no withdrawal times, and the inclusion rate

recommended for cattle is 0.25% to 1% of the diet DM.

Application of Tasco to plant and its effects on plants and animals. Studies have

demonstrated benefits of Tasco supplementation for plants and animals. As a fertilizer of

soil and plants, Tasco may improve utilization of minerals, growth and immunity. Foliar

application of the seaweed extract increased growth and also the concentration of α-

tocopherol, β-carotene, ascorbic acid and superoxide dismutase in tall fescue and

Kentucky bluegrass (Zhang, 1997). Moreover, a quick and long-lasting increase in

superoxide dismutase activity was observed in endophyte-infected and non-infected tall

fescues, with no effect on composition of fiber, crude protein, total non-structural

carbohydrates, and in vitro dry matter digestibility (Fike et al., 2001). The increased

antioxidant activity, coupled with the presence of plant growth regulators, such as

cytokinin, may improve plant resistance to stress (Allen et al., 2001b).

Application of Tasco to plants may also indirectly affect animals consuming the

plant. A study with wether lambs grazing endophyte-infected tall fescue treated with

Tasco-Forage during summer, revealed a linear increase in gain in response to Tasco

concentration (Fike et al., 2001). Moreover, serum vitamin A and whole-blood Se tended to be higher in lambs and steers grazing Tasco-treated tall fescue (Fike et al., 2001). The authors speculated that the positive effects of Tasco were related to the increase in antioxidant activity in the plant, such as superoxide dismutase, which improved the immune system and stress tolerance of plants and animals.

In another study, application of Tasco to infected tall fescue improved immune function, by improving phagocytic and major histocompatibility complex (MHC) class II activity, of steers on pasture (Saker et al., 2001) and later on during the finishing phase (Allen et al., 2001a). However, when Tasco was applied to non-infected tall fescue, no effects were observed on the immune system of steers during the grazing period (Saker et al., 2001). However, this effect was reversed during the finishing phase, after animals were exposed to the stress of transportation to the feedlot, indicating Tasco's effect on immune function is dependent on the presence of a stressor (Allen et al., 2001a). Alkaloids present in tall fescue may cause oxidative stress in animals, by affecting growth hormone, prolactin, dopamine and cytokines activity, altering free radical production, behavior and thermoregulatory ability by animals (Saker et al., 2001). In this study, Tasco was found to improve monocyte cell function and innate immunity in steers grazing infected tall fescue; this effect may be related to an increase in antioxidant activity in the plant ingested by the animal, or directly in the animal organism. Tasco had no effect on weight gain, during the grazing period (Saker et al., 2001), or on performance, during the finishing phase (Allen et al., 2001a).

Most studies evaluating the effects of Tasco during heat stress are related to fescue toxicosis, which is known to cause hyperthermia. Tasco-Forage treatment of

26

infected tall fescue was shown to sporadically lower rectal temperature of steers on pasture, when compared to untreated forage (Saker et al., 2001). However, in the finishing phase, steers grazing Tasco-treated forage had a greater rectal temperature than those grazing untreated pasture, after 28 and 56 days in the feedlot; no differences were seen prior to or after these times (Allen et al., 2001a). The authors speculated that these findings may be related to altered thermoregulatory mechanisms caused by Tasco, and they further suggested that it is not related to thyroid function alteration (Allen et al., 2001a). Other authors speculated that some of the effects of Tasco on temperature regulation in animals may be attributed to cytokines, which may affect body temperature and feed intake (Allen et al., 2001b). It is hard to determine the validity of the body temperature responses seen for both experiments, since only one measurement was obtained every 28 days, which is not an accurate representation of the animal's response.

Tasco supplementation to animals: performance and thermal status. The direct influence of Tasco supplementation on animals has also been evaluated. Eichen et al. (2001) found inclusion of 1% Tasco in the diet containing endophyte-infected tall fescue seed reduced core body temperature of male rats during heat challenge. Spiers et al. (2004a) studied rats and steers fed endophyte-infected tall fescue seed and supplemented with Tasco-EX. Tasco-EX reduced core body temperature of rats exposed to heat stress but it had no effect on feed intake or growth (Spiers et al., 2004a). In contrast, steers fed Tasco at the 1% level had lower DM intake than animals in the control group or those fed Tasco at 0.5% level, after approximately one week of heat exposure (Spiers et al., 2004a). Furthermore, steers consuming 1% Tasco in the diet had temporarily lower rectal temperature and respiration rates than control, suggesting Tasco fed at 1% reduce

27

hyperthermia caused by fescue toxicosis in cattle, during heat stress periods (Spiers et al., 2004a). According to the authors, the reduction in core body temperature may be attributed to a decrease in metabolic heat production or an increase in the ability for evaporative heat loss other than by increased respiration rate, since it was decreased along with rectal temperature. Also, since feed intake was reduced in those steers on the 1% Tasco treatment, it was suggested that the reduction in energy intake may have reduced heat production (Spiers et al., 2004a).

Williams et al. (2009) studied the effects of 1% Tasco-14 supplementation on steers housed in climatic chambers and exposed to heat stress. The study revealed that steers on Tasco had a lower range of rectal temperature and a higher range of respiration rate compared to the control treatment, during early exposure to heat. However, the authors suggested that the greater respiration rate range during that time was not sufficiently high to cause the reduction of rectal temperature by increased respiratory evaporation. In this study, Tasco had no effect when steers were exposed to higher levels of heat load. Steers that were exposed to heat stress after being fed Tasco for 36 days at thermoneutrality had higher rectal temperature and range of rectal temperature, lower maximum respiration rate, and lower range of respiration rate, compared to the control treatment (Williams et al., 2009). This suggests Tasco fed for a longer period may decrease its effects on reducing core body temperature under heat stress. The authors suggested that a lower dose or a lower length of feeding Tasco may have better results in lowering rectal temperatures (Williams et al., 2009). In this same study, there was a tendency for a lower DM intake of steers consuming Tasco during early exposure to heat, with no effects on final weight and ADG (Williams et al., 2009). Supplementation with

28

Tasco improved fiber digestibility by enhancing *in situ* DM and *in situ* NDF disappearances during early exposure to heat stress, with no negative effect on rumen pH, rumen ammonia concentration, and particulate passage rate (Thompson, 2004).

In a subsequent trial, Thompson (2004) studied possible carry-over effects of Tasco, by transferring those steers from chambers to pasture, where they grazed endophyte-infected or endophyte-free tall fescue during summer. Steers grazing infected tall fescue that were previously fed 1% Tasco-14 for 40 days presented higher total gain and 80% greater ADG compared to animals that had grazed infected tall fescue without previously receiving Tasco (Thompson, 2004). However, during the finishing phase, no effect of previous supplementation with Tasco was observed on total gain and ADG (Thompson, 2004). Tasco reduced by 0.3°C the core body temperature of steers grazing infected fescue during early heat exposure, reaching levels of body temperature relative to animals on non-infected pasture (Spiers, 2003). Moreover, Tasco appeared to delay the initial increase in core body temperature of steers grazing infected fescue compared to the control treatment, as a response to the increase in air temperature (Spiers, 2003).

Another study was conducted to evaluate the benefits of *A. nodosum* in lambs exposed to transportation stress for 12 hours in hot weather (Archer et al., 2007). The results showed that the seaweed lowered maximum and average ear canal temperature below the control levels when fed at a rate of 2% of DM intake per day for 2 weeks (Archer et al., 2007). Also, this treatment resulted in the smallest change in ear canal temperature from the beginning to the end of the transport period. Body weight lost, as an effect of transport, was not affected by treatments. The authors found that the 2%

inclusion rate was the most adequate to reduce ear canal temperature of lambs exposed to the stress of transportation during heat stress (Archer et al., 2007).

Another aspect of the influence of Tasco on the animal's well being is the effect on the immune system. Saker et al. (2004) evaluated the effects of Tasco on growth, and both immune and oxidative stress responses of lambs exposed to 10 days of heat stress, after being fed dietary treatments for 27 days. Dietary treatments consisted of endophyte-infected tall fescue hay untreated- (control) or treated- pre-harvest or post-harvest (directly fed) with Tasco. Animals fed pre-harvest-treated forage had a lower final body weight than animals directly fed Tasco, with the control group being intermediate. Although DM intake was not presented, the authors suggested that feeding Tasco directly may improve palatability or nutrient value of hay (Saker et al., 2004). Moreover, feeding Tasco in both pre- and post-harvest forms improved antioxidant status of animals compared to control, alleviating the negative effects of heat stress on immune system. However, feeding Tasco directly appeared to further improve immune status of lambs, with higher phagocytic capacity, capacity for oxidative burst, and superoxide dismutase activity, and lower lipid peroxidation (Saker et al., 2004).

Effects of Tasco on carcass traits. The influence of Tasco on carcass quality has also been reported. One study demonstrated that supplementation of implanted cattle with A. nodosum improves quality grade, with the best inclusion rate being 2% Tasco for 14 days in the beginning of finishing phase (Anderson et al., 2006). At this level, A. nodosum improved marbling score, USDA quality grade, and firmness of lean muscle, with no effects on animals performance (weight gain, ADG, or feed efficiency) during the finishing phase, indicating that the seaweed did not impact the efficiency of implants

(Anderson et al., 2006). These findings were confirmed by other studies (Allen et al., 2001a; Braden et al., 2007). Steers fed Tasco at 2% DM, during the beginning and ending of the finishing phase, had higher marbling score and USDA quality grade, lower preliminary yield grade, less discoloration, and more redness color during extended retail periods (Braden et al., 2007). No effects of Tasco were seen in longissimus muscle area, hot carcass weight, KPH (kidney, pelvic and heart fat), final yield, and sensory attributes (Braden et al., 2007). In another study, steers previously grazing endophyte-infected tall fescue treated with Tasco-Forage had an improvement in carcass quality, with enhanced USDA quality grade and marbling score, while hot carcass weight was not affected (Allen et al., 2001a). Moreover, meat from animals that grazed tall fescue treated with Tasco had an extended shelf-life and better color stability during the retail display and no negative effect of Tasco was observed on sensory characteristics (Montgomery et al., 2001). The authors suggested that the effects of Tasco were long-lasting, since differences in carcass characteristics in steers were observed for about 160 days after removal from the treated pasture (Montgomery et al., 2001). The improvement in meat quality may be attributed to better fat metabolism and antioxidant activity, reduction of stress and enhancement of immune system and health, resulting in greater energy availability for production functions and fat deposition, suggesting that Tasco may be used as a supplement to improve meat quality (Allen et al., 2001a; Anderson et al., 2006; Saker et al., 2001).

Effects of Tasco on microorganisms and diseases. Studies have shown that Tasco may be effective in reducing pathogenic microorganisms responsible for infections as well as problems associated with the diseases. A swine study evaluated the

31

antimicrobial properties of A. nodosum. Pigs were fed the seaweed for 14 days followed by an enteric disease challenge with Salmonella typhimurium (Turner et al., 2002). The seaweed treatment had little effect on overall growth, but a quadratic effect was seen in ADG and final weight, with an inclusion rate of 1% A. nodosum being the most effective (Turner et al., 2002). However, the increasing inclusion rate of A. nodosum in the feed linearly increased ADFI and decreased G/F, representing a negative impact on feed efficiency (Turner et al., 2002). Another study evaluated the effects of Tasco, fed for 14 days prior to slaughter, on reduction of fecal shedding of feedlot cattle (Behrends et al., 2000). There was a linear decrease in Escherichia coli count with increasing levels of Tasco supplementation and a decrease in Escherichia coli O157 for animals which received the seaweed; no differences were found for Salmonella count (Behrends et al., 2000). In another study, supplementation with 2% DM of Tasco, 14 days prior to slaughter, also showed a reduction in Escherichia coli O157 and Escherichia coli O157:H7 in fecal shedding and hide swabs sampled from feedlot steers and heifers (Braden et al., 2004). The antibacterial activity of A. nodosum extract was tested against 11 organisms, including pathogenic Gram positive and negative bacteria (Vacca and Walsh, 1954). The seaweed was shown to be effective against all microorganisms tested, including E. coli, except for one organism (Bacillus subtilis) (Vacca and Walsh, 1954). Some authors speculated that the antibacterial activity against E. coli is more likely to be attributed to some alteration in the GI-tract rather than in the immune function (Archer, 2005), although other authors did not find differences in fecal pH and VFA concentration to justify that claim, when testing the effect of Tasco in reducing fecal shedding in steers inoculated with Escherichia coli O157:H7 (Bach et al., 2008). In either case, the

reduction in E. coli could reduce or prevent carcass contamination at the slaughter house, contributing to food safety.

Active ingredients in Tasco

In an attempt to identify the active components of *A. nodosum* responsible for the effects on body temperature and immunity observed in previous studies, Archer et al. (2008) fed fucoidan, salt (sodium chloride and potassium gluconate) or betaine to lambs exposed to stress of transportation during hot weather. Salt treatment had a similar response, with increased white blood cell and lymphocyte counts, as the seaweed treatment, which could improve cellular immunity and reduce animal infections (Archer et al., 2008). In contrast to previous findings by Burtin (2003), Archer et al. (2008) found no effect of fucoidan on immune system. Betaine was tested in this experiment as a possible factor having the same effects as *A. nodosum* in body temperature. However, neither betaine or any other substance tested had a similar effect on body temperature, even though the only effect of *A. nodosum* was a smaller range of ear canal temperature during transport, with no differences on minimum, maximum or average ear canal temperature (Archer et al., 2008). Other authors have speculated that the benefits from the seaweed may be related to the high amount of iodide present, which could lower thyroid activity and metabolic heat production, lowering core body temperature (Spiers et al., 2004a). However, a study evaluating the inclusion of Tasco or iodide in the diet of rats showed that not all of the effects of Tasco were related to its iodide content (Eichen et al., 2003). Another substance in Tasco might have an influence. Thus, it is still unknown what ingredient in Tasco may be responsible for the observed effects.

Tasco supplementation for dairy cows

Few studies have evaluated the effect of *A. nodosum* on dairy cows performance, especially when exposed to heat stress. To the best of our knowledge, no publications are available at this point, besides some research trials. A trial was performed at University of Arkansas to evaluate the effects of Tasco on large and small cows exposed to heat stress during the months of June through September (Kellogg et al., 2006). The experiment revealed that Tasco supplemented at 0.25% of diet prevented a decline in milk production during heat stress, especially for larger cows, which are more susceptible to heat stress because of the higher metabolic rate (Kadzere et al., 2002). Somatic cell score was lower for cows fed Tasco, but only during August; cows on Tasco treatment had lower respiration rate but only for some days (Kellogg et al., 2006). A study at Kansas State University during summer heat stress indicated a higher milk production and higher amount of milk protein in cows fed 56.7 g of Tasco-14 per day, while no differences in DM intake were found, suggesting that Tasco may increase lactation efficiency; no effects were seen for respiration rates, rectal and rear udder skin temperature (Cvetkovic et al., 2005).

SUMMARY

The negative impact of heat stress in dairy production, associated with reduced performance, health and well-being, and increased mortality have been discussed. Moreover, physiological responses of dairy cows to adverse weather conditions during heat stress periods have been defined. These alterations include thermal responses, such as increased respiration rate and body internal and surface temperatures, impacting

performance parameters, like feed intake and milk production. Means to alleviate heat

stress effects on dairy cows include environmental modification, management strategies

and nutritional alterations. Supplementation of diets with feed additives, such as seaweed

(Tasco, *Ascophyllum nodosum*), have been demonstrated to alleviate the negative impact

of hot weather, by reducing core body temperature (Archer et al., 2007; Spiers et al.,

2004a) and improving immune function, without affecting performance (Allen et al.,

2001a; Fike et al., 2001; Saker et al., 2001). However, more research is needed in order to

identify specific effects of Tasco supplementation for dairy cows exposed to heat stress.

Table 1.1. Composition of Tasco[1] (*Ascophyllum nodosum*) meal, utilized for livestock

Item	Value
Crude fiber, %	6.0
Carbohydrates,%	52.0
Ash, %	22.0
Moisture, %	12.0
Crude protein, %	6.0
Minerals	
Aluminum, ppm	20–100
Arsenic, ppm	< 3
Calcium, %	1.0–3.0
Copper, ppm	4–15
Iodine, ppm	< 1,000
Magnesium, %	0.5–1.0
Manganese, ppm	10–50
Phosphorus, %	0.1–0.2
Potassium, %	2–3
Selenium, ppm	< 1
Sodium, %	2.4–4.0
Sulphur, %	2.0–2.3
Zinc, ppm	35–100
Amino acids, g/100 g of protein	
Alanine	5.3
Arginine	8.0
Aspartic acid	6.9
Cystine	trace
Glutamic acid	10.0
Glycine	5.0
Histidine	1.3
Isoleucine	2.8
Leucine	4.6
Lysine	4.9
Methionine	0.7
Phenylalanine	2.3
Proline	2.6
Serine	3.0
Threonine	2.8
Tyrosine	0.9
Valine	3.7

[1]Acadian Seaplants Limited, Nova Scotia, Canada
Adapted from Allen et al. (2001b)

CHAPTER 2

IMPACT OF TASCO ON ALLEVIATION OF HEAT STRESS IN DAIRY COWS

ABSTRACT

The present study determined the impact of Tasco-14, *Ascophyllum nodosum*, on heat stress in dairy cows. Thirty-two Holstein cows were blocked by parity and DIM and assigned to treatments using a randomized complete block design, with 8 cows per treatment. The study was divided into 3 periods. In Period 1 (7 days), cows adapted to the Calan Gate system. In Period 2 (28 days) the following treatments began: Control-1 (C-1); Control-2 (C-.5T); 0.25% Tasco (0.25T); 0.50% Tasco (0.5T). In Period 3 (28 days), C-.5T was changed to 0.50% Tasco in order to evaluate duration of feeding Tasco. Cows were fed a TMR diet, consisting of corn silage/alfalfa haylage and corn. Feed intake and milk production were recorded daily. Core body temperature (T_{core}) was recorded every 20 minutes with telemetric temperature transmitters (SmartStock, Pawnee, OK) placed in the reticulum. Respiration rate (RR), measured by enumeration of abdominal movements for 30 seconds, and rump (T_{rump}) and ear (T_{ear}) skin temperatures, measured with an infrared heat gun (Raytec, Everett, WA), were collected daily at 0700, 1600, and 1900 h. Ambient temperature (T_a) and relative humidity were recorded (Hobo, Onset Computer

Corp., Bourne, MA) in 15 minute intervals. For Periods 1, 2 and 3 the average maximum T_a was 29.6, 28.0 and 31.0°C, respectively. In Period 2, no differences ($P > 0.10$) were found between C-1 and C-.5T for any parameter, so they were combined for this period (C). Dry matter intake (DMI) was occasionally lower ($P < 0.05$) for 0.25T compared to other groups, but no differences ($P > 0.10$) in milk production were observed. At 1600 and 1900 h, 0.25T tended to have lower T_{ear} vs. 0.5T ($P < 0.10$) and lower T_{rump} vs. other treatments ($P < 0.01$). In Period 3, no differences ($P > 0.10$) in DMI and milk production were noted. Differences in T_{rump} ($P < 0.05$) were observed between treatments but no specific pattern could be distinguished; 0.25T had occasionally lower T_{rump} compared to other treatments. In Periods 2 and 3, a trend existed ($P < 0.10$) for higher T_{core} for 0.5T compared to other treatments, but the differences found were more likely attributed to animal individual differences than to treatment differences. In Period 2, linear regression of body core and surface temperatures vs. T_a revealed 0.25T had a lower ($P < 0.05$) increase in T_{core} with the rise in T_a during the day compared to C; in Period 3, both 0.25T and 0.5T had a lower ($P < 0.05$) increase in T_{core} compared to C-1 and C-.5T, and additionally, 0.25T had a lower increase in T_{rump} ($P < 0.05$) and T_{ear} ($P < 0.10$) compared to C-1 and C-.5T. From the results, it suggests the inclusion of 0.25% Tasco may reduce heat strain of cows, maintaining lower body core and surface temperatures with increasing T_a, and sustaining milk production, even with a reduction in DMI; however, no progressive benefit was seen with the increase of Tasco concentration in the diet. No effects of Tasco were seen for any parameter when fed only during Period 3, for 28 days.

INTRODUCTION

Heat stress is one of the main causes of economic losses in livestock production in the United States, caused by decreased performance (production, efficiency and reproduction), health and well-being, and increased mortality of animals. St-Pierre et al. (2003) estimated losses of $ 2.4 billion dollars per year for the livestock industry, with the dairy industry contributing $ 1.5 billion dollars. Lactating dairy cattle are one of the most affected animals because of the large amount of metabolic heat produced (Kadzere et al., 2002), added to the heat accumulated by radiant energy and the difficulty in dissipating body heat to the environment, when ambient conditions are unfavorable (i.e. high ambient temperature and relative humidity) (West, 2003). Several approaches may alleviate heat stress in dairy cows, including management and feeding strategies, as well as the use of feed additives. Although no feed additives have effectively reduced heat strain, there is some evidence that brown seaweed (*Ascophyllum nodosum*) may reduce core body temperature for the short-term (Archer et al., 2007; Spiers et al., 2004a) and improve immune function during heat stress, with no negative effect on performance (Allen et al., 2001a; Fike et al., 2001; Saker et al., 2001). Previous work (Spiers et al., 2004a; Williams et al., 2009) showed that Tasco-14 fed at 1% of diet DM had a short-term effect in lowering internal body temperature of beef steers exposed to heat stress. Thus, the objective of this study was to evaluate the ability of Tasco, *Ascophyllum nodosum,* to alleviate the effects of heat stress in dairy cows, based on production traits (feed intake and milk production) and animal responses (respiration rate, skin and core body temperatures), under fluctuating ambient temperature and humidity conditions.

MATERIALS AND METHODS

Animals and dietary treatments

A study was performed utilizing 32 Holstein dairy cows (average milk production = 33.6 ± 4.8 kg/d, ranging from 25.2 to 44.0 kg/d) during summer 2008, for 63 days, from June 2 to August 3. Animals were housed in a free-stall barn at the University of Missouri Foremost Dairy Research Center (Columbia, MO). The Animal Care and Use Committee at the University of Missouri approved the protocol for conducting this study. Cows were blocked based on parity (2.7 ± 1.5; ranging from 1 to 6) and days in milk (138 ± 43 days; ranging from 56 to 208) and assigned to one of four treatments (trt), with 8 cows per treatment. The study was a randomized complete block design and divided into 3 periods. The first period (June 2 to June 8; 7 days) was an adjustment period to the Calan Gate system. During the second period (June 9 to July 6), lasting 28 days, the cows started the dietary treatments, while in the third period (July 7 to August 3), also lasting 28 days, one of the dietary treatments was changed. In Periods 2 and 3, treatments were: 1. Control (C-1); 2. Control + 0.50% Tasco (C-.5T); 3. 0.25% Tasco (0.25T); 4. 0.50% Tasco (0.5T). In Period 2, C-1 and C-.5T received the same control TMR diet, consisting of corn silage/alfalfa haylage and corn, while 0.25T and 0.5T received the TMR supplemented with 0.25% and 0.50% of Tasco, respectively. In Period 3, the only treatment changed was C-.5T which became a second treatment of 0.50% Tasco. The intent of adding another 0.50% Tasco treatment was to evaluate the effects of Tasco being fed for 28 vs. 56 days to dairy cows on core and skin body temperatures, respiration rate, feed intake and milk production.

Feeding management

Cows were fed individually twice daily at 0800 and 1400 h and feed intake was recorded; weigh-backs were recorded the following morning to correct for daily feed intake. Tasco was top-dressed onto the TMR during the morning feeding, and the amount added was calculated based on the average DMI of three previous days. Tasco was mixed with 100 g of DDG, before added to the TMR, to avoid selectivity by the animals. Cows in control groups also received 100 g of DDG, in order to maintain the same basic diet. In Period 2, 0.25T and 0.5T consumed daily an average of 63.0 g and 131.9 g of Tasco, respectively, while in Period 3, C-.5T, 0.25T, and 0.5T received daily an average of 114.9 g, 55.7 g, and 117.4 g of Tasco, respectively. Animals were weighed at the beginning and end of the study, and also once a week, to determine gain. Body condition score (BCS) was recorded at the same period, using a 1 to 5 scale, 2.5 being the ideal score.

Milking management and sample collection

Cows were milked twice daily at 0500 and 1700 h, following procedures recommended by the National Mastitis Council (2002). Milk production of each cow was recorded electronically at each milking (Metatron 12; Westfalia, Elk Grove Village, IL). Morning and afternoon production were combined to obtain a daily milk production. A milk sample from each cow was collected once a week in two consecutive milkings and preserved with bronophol (Broad Spectrum Microtabs II; D and F Control Systems, Inc., Dublin, CA). Samples were analyzed for protein, fat, and somatic cell count (SCC) by Mid-South Dairy Records Laboratory, Springfield, MO.

Analytical procedures

      Feed sample analysis. Feed samples were collected daily and analyzed for DM (AOAC, 1995). Feed was combined by week and two samples for each week were collected. Each weekly sample was ground through a Wiley Mill (Thomas Scientific, Swedensboro, NJ) using a 2 mm screen, and analyzed in duplicate for DM (AOAC Official Method 934.01, 1995), NDF and ADF (Van Soest et al., 1991). Total nitrogen was determined by combustion analysis (LECO Instruments, Inc., St. Joseph, MI; AOAC Official Method 990.03, 1995) and the CP was obtained by multiplying the total nitrogen by 6.25. The composition and chemical analysis of the TMR diet are shown in Table 2.1.

      Milk sample analysis and milk efficiency calculations. Milk samples were analyzed for protein and fat percent (Bentley 2000, Bentley Instruments, Chaska, MN) and SCC (Somacount, Bentley Instruments, Chaska, MN). The results for protein and fat percent of each milking were averaged to obtain a weekly value. Also, protein and fat percent of each milking were multiplied by the correspondent milk production value, and the two calculated yields were then summed, obtaining one weekly value for the yield milk component. For SCC, linear score was calculated through the formula: SCC linear score $(SCS) = \log_2 (SCC/100{,}000) + 3$. Scores lower than 5 (400,000 cells) represents healthy cows; clinical mastitis is identified by scores greater than 5.9 (750,000 cells), while subclinical mastitis is represented by score from 5 to 5.9.

      Energy-corrected milk (ECM) was calculated daily through the formula: ECM = (0.327 x milk yield [kg/d]) + (12.95 x fat yield [kg/d]) + (7.2 x protein yield [kg/d]) (Orth, 1992). Since milk production and feed intake were recorded daily, while fat and protein data were collected weekly, estimations of daily protein yield and daily fat yield

were calculated by multiplying the weekly value of the component by the daily value of milk production, during the respective week. The ECM calculated for each day was divided by the DMI value of the corresponding day, in order to represent the milk efficiency, which was compared among treatments.

Thermal status measurements

      Respiration rate and skin surface temperatures. Thermal status measurements (skin temperature and respiration rate) were taken daily at 0700, 1600, and 1900 h. Skin temperature was measured using a calibrated infrared heat gun (Raytec, Everett, WA), in two different locations, rump (T_{rump}) and ear (T_{ear}), which were previously shaved. Respiration rate was measured by enumeration of abdominal movements for 30 seconds.

      Core body temperature. Core body temperature (T_{core}) was recorded using telemetric temperature transmitters (SmartStock, Pawnee, OK) inserted orally using a bolus gun, to lodge in the reticulum of each cow. The transmitters were approved by FDA and do not need to be removed. The telemetric system was composed by a bolus, antenna, barn receiver unit, base receiver unit, and a computer, installed with a software program for logging the data, and located inside a building, about 30 meters away from the free-stall barn. The antenna for transmitting data to the base receiver unit was located in the center of the barn where cows were housed, above the free-stalls, about 3 meters of the floor. The telemetric bolus (3 cm in diameter, 8.5 cm in height, and 120 g in weight) was programmed to record temperature every 20 minutes, with each transmission containing 12 readings. A maximum T_{core} value per hour was obtained from the 3 recordings in order to eliminate the variation in reticulum temperature due to the influence of water consumed by the animals. It has been shown that rumen temperature

(i.e. T_{core} in this study) is influenced by water consumption of the animals. Beef cows

drinking water at $16^{o}C$ had a decrease in rumen temperature ranging from 0.5 to $3.6^{o}C$,

and the maximum decrease happened 30 minutes after water consumption, lasting for

more than 2 hours (Boehmer et al., 2009). Each day of the study consisted of 24 T_{core}

values representing every hour. Certain days were removed from the data set due to loss

of data for entire or part of the day. The loss of data was attributed to a power outage, due

to an electrical storm. This problem occurred on days 1, 3, 11, 12, 23, 50, 51, 58, 59, and

62, and so, these 10 days were removed from the data set for T_{core}. Moreover, a test for

outliers was performed; a quadratic regression of T_{core} vs. T_a was developed for each

animal and all T_{core} observations that were greater than 2.5 standard deviations from the

predicted value were removed from the data set. This new data set, with the outliers

removed, was utilized for all the analysis of T_{core} in this study. One cow from C-1 was

removed from the whole study for the T_{core} analysis, since the transmitter was not

accurate.

For the analysis of T_{core}, days were tested with the 24 hours (whole day) and also

with two ranges of hours: from 0 to 0600 h (nighttime) and from 0700 to 1600 h

(daytime), which were defined by visually observation of T_{core} pattern as an average of all

days in the study (Figure 2.1). These ranges of time were used to evaluate the influence

of treatment on the cow's T_{core} response to the drop (nighttime) and rise (daytime) in T_a

throughout the day. These two ranges of hours, as well as the entire 24-h day, were

utilized for the statistical analyses of T_{core}. Ideally, these ranges should avoid the milking

periods (from 0500 to 0700 h and from 1700 to 1900 h), since the cows were moved to

the milking parlor, in which T_a was not recorded. For daytime, the peak in T_{core} occurred

between 1400 and 1600 h, so the range was ended before the cows were moved to the parlor at 1700 h. In fact, the highest T_{core} occurred around 2200 h (Figure 2.1); however, 1600 h was chosen to be the end of the daytime period, since a major drop occurred after 1700 h when the cows were moved to the parlor. For the nighttime, T_{core} did not drop significantly from 0 to 0400 h; the major drop was observed between 0500 and 0600 h when cows were taken to the milking parlor (Figure 2.1). So, the nighttime was included with the morning milking in order to visualize the major drop in T_{core} of the day. With that, it was possible to evaluate T_{core} among treatments; however, it was not possible to compare T_{core} with T_a, since ambient conditions of the milking parlor were not collected.

Because there was a drastic drop in T_{core} when cows were moved to the parlor, it was necessary to determine if the drop was natural, or if it was due to something else, for example, a lower T_a in the parlor compared to the T_a in the barn. Since ambient conditions were not collected in the parlor during the study, it was necessary to verify it during the next summer. Thus, during the months of June, July and August 2009, data loggers were placed in the barn (in one of the three positions that were utilized the past year) and in the parlor. By comparing the temperature records for the two locations (Figure 2.2), it was noticed that the T_a in the parlor may be lower for some hours (from 0900 to 1600 h), but during the milking times (morning and afternoon) the T_a in the parlor is much higher than the temperature in the barn, because of the presence of the cows that are producing heat. Since the cows in our study were not the first ones to be milked, there was no reason to relate the drop in T_{core} with the lower T_a in the parlor, because the T_a in the parlor was probably already elevated when the cows entered the facility. However, one explanation for the decrease, besides a natural drop in T_{core} during

45

early morning, is that cows had an opportunity to drink from water troughs on the way from the barn to the parlor; so, the cows probably drank a large amount of water as they entered the parlor, contributing to the decrease in T_{core}. Other authors also found a decrease of about 0.3ºC of vaginal temperature of dairy cows at milking time, even in dry cows, which were only walked through the parlor to simulate the milking (Araki et al., 1984). However, when the dry cows were not walked to the parlor, the same drop at about the same time was observed. The authors suggested that the drop may be attributed to the pre-milking wash of the udder or the shade in the parlor (cows in that experiment were housed in unshaded pens) (Araki et al., 1984). However, it was not understood why dry cows not walked through the parlor had the same drop in body temperature.

After analyzing T_{core} during Periods 2 and 3, it was noticed that the 0.5T treatment had generally higher T_{core} than other groups. In several observations, differences among treatments were attributed to the higher T_{core} of the 0.5T. Thus, in order to remove any animal effects and express true treatment effects, differences in T_{core} from a baseline, utilizing data from Period 1, could be analyzed. The baseline represented an average of days in Period 1 (d 1 to 7; or d 5 to 7; or d 6 and 7), during the nighttime, daytime or whole day. All these different approaches were tested in order to identify the best way to obtain a baseline for the study. These baselines were tested for treatment differences and trt x h interaction, in order to identify any possible differences between pre-assigned treatments. If differences between pre-assigned treatments were found, the data set for T_{core} would be replaced by the change in T_{core} for each cow, obtained by the subtraction of each data point collected during Periods 2 and 3 from the proposed baseline. With that,

46

differences between pre-treatments would be minimized and true differences between treatments would be better visualized.

Ambient conditions measurements

Ambient temperature (T_a) and relative humidity (RH) were automatically recorded every 15 minutes using data loggers (Hobo, Onset Computer Corp., Bourne, MA), which were placed in three different locations in the barn. Two of the loggers were placed in the center of the barn, above the free-stalls, about 3 meters of the floor; the other logger was placed in the mid-point above the Calan Gate feeding system, 3 meters of the floor. Ultimately, an average T_a of the three data loggers was obtained in order to represent an hourly average T_a in the barn. Two of the three loggers presented errors in the measurement of RH, so, only one was utilized to represent the humidity in the barn. Temperature-humidity index (THI) was calculated hourly by the formula: THI = (T_a x 0.81) + RH(T_a – 14.4) + 46.4, where T_a is the dry-bulb temperature (ºC), and RH is the relative humidity (decimals) (Thom, 1959). Additionally, T_a and RH were recorded at the same time RR and skin temperatures were collected, at 0700, 1600 and 1900 h.

Data analysis

For all parameters analyzed, only healthy animals were utilized. Cows with mastitis for a certain period during the study were removed from the data set for that specific period of sickness and placed back into the group after they recovered. In treatment C-.5T, one cow had persistent mastitis toward the end of the study and was removed from Period 3; she also had mastitis in the first two days of the study, and was removed then. Three other cows in the same treatment had mastitis for a period of time and were removed for d 13 to 18, d 28 to 41, and d 54 to 59. In treatment 0.25T, one cow

had mastitis from d 36 to 43, and for treatment 0.5T, one cow was sick during d 23 through 27, and they were removed for those respective days.

The relationship between cow thermal status and ambient conditions was evaluated to determine the influence of Tasco on body core and surface temperatures as ambient temperature increased. The THI (or RH) were not included in the analysis because T_a and THI were highly correlated for all the periods ($R^2 = 0.97$ for Period 1, and 0.94 for Periods 2 and 3), indicating that T_a alone could describe effectively the ambient conditions. For T_{rump} vs. T_a, the quadratic regression was not significant for any treatment in Periods 2 and 3, so the linear regression was utilized to describe the relationship. For T_{ear} vs. T_a, the quadratic regression was not significant for any treatment in Period 2, but the opposite occurred in Period 3, when the quadratic function was significant for all treatments. However, the 95% confidence interval (CI) of the linear and quadratic curves were coincident for all cases, indicating that the linear regression may be utilized without losing quality of the analysis.

For T_{core} vs. T_a, the relationship was analyzed for the daytime, since both the whole day and nighttime ranges included some hours when cows were in the milking parlor, where ambient data were not monitored. Average T_{core} was obtained for each treatment and T_a value within a period. In other words, for the same value of T_a, T_{core} of each cow were averaged to get one mean T_{core} for each T_a. Linear and quadratic regressions were tested. For most of the cases, the relationship between T_{core} and T_a was quadratic, even though for some cases the linear function could appropriately describe the relationship. From the quadratic regression between T_{core} and T_a, two linear curves were obtained as shown in Figure 2.3. "Linear 1" represented T_{core} at thermoneutrality (lower

T_a values), while "Linear 2" represented T_{core} at heat stress (higher T_a values). The intersection between Linear 1 and Linear 2 was determined by the highest R^2 for each individual line, obtained by testing the addition of points to the data set, one at a time. After determining the intersection for each treatment in each Period, two new data sets were formed for each treatment, one consisting of data points within the Linear 1 set, and the other containing data points in the Linear 2 set. Linear 1 was assumed to have a non-significant slope, since T_{core} is poorly correlated to T_a at thermoneutrality (Berman et al., 1985), and so, data sets within Linear 1 were tested for differences between treatment means. On the other hand, Linear 2 was expected to have a significant slope, and so, linear regression between T_{core} and T_a were performed for data sets within Linear 2 to determine differences between treatment slopes.

The data were analyzed as a complete block design with repeated measurements over time (Littell et al., 1998), for each period of the study, and included RR, T_{ear}, T_{rump}, T_{core}, DMI, and milk production and composition as influenced by Tasco. Within each period, main effects for all the parameters, as well as two and three-way interactions, were analyzed using LSMEANS function of MIXED procedure of SAS (SAS Institute Inc., Cary, NC). The main effect of T_{core} was analyzed as daily minimum, maximum and change (difference between maximum and minimum), as well as the average for nighttime and daytime ranges. The influence of T_a on the thermal status was analyzed using PROC REG of SAS, with linear or quadratic function; linear regressions were utilized rather than quadratic if it sufficed to express the relationship. Slopes were obtained for each treatment and compared by PROC GLM of SAS to determine differences. The BW change and body condition score change were analyzed as a

randomized complete block design. The linear model contained the main plot effects of Tasco, time, and the interaction of Tasco x time. Animal was the experimental unit, and value of $P < 0.05$ was utilized for establishing significant differences, while $P < 0.10$ was pointed as trends. Figure 2.4 presents a summarized description of the study.

RESULTS AND DISCUSSION

Period 1

Period 1 consisted of the first 7 days of the study, when cows acclimated to the Calan Gate system. Dietary treatments did not start until the beginning of Period 2.

Ambient conditions for Period 1. In Period 1 (Figure 2.5), the average daily values for maximum, mean and minimum T_a were 29.6, 26.0 and 21.7ºC, respectively, while the maximum and mean RH were 91.8 and 79.0%, respectively. For all days during this period, except for d 1, maximum T_a was greater than 29ºC. The highest T_a was observed at 1400 h on d 7, reaching 30.8^oC. A T_a of 25ºC can be considered as a threshold for heat strain in lactating dairy cows (Berman et al., 1985). The elevated T_a for Period 1 may have caused some heat strain for the cows during this baseline period.

Performance parameters for Period 1. All parameters analyzed during the study were measured during this period and examined for pre-assigned treatment differences. No differences ($P > 0.10$) were found among pre-assigned treatments for DMI, milk production and components, milk efficiency, BW change or BCS change (Table 2.2).

Thermal status for Period 1: Respiration rate and skin temperatures. The average RR and skin temperatures per treatment for each time a day when data were measured, as well as the daily average for the three measurements are presented in Table

2.3. No differences ($P > 0.10$) were found among pre-treatments for RR, T_{rump}, and T_{ear} for main effect or interaction (trt x d, trt x h or trt x d x h).

Thermal status for Period 1: Core body temperature. The results for the analysis of T_{core}, for the whole day, nighttime and daytime are shown in Table 2.3. No differences ($P > 0.10$) were observed among treatments for minimum, maximum, change or during nighttime and daytime ranges. When testing baselines for Period 1, it was found that no differences ($P > 0.10$) existed between pre-treatments and that the interaction trt x h was not significant ($P > 0.10$) for any of the approaches (varying range of hours and number of days during Period 1), suggesting that, even though 0.5T had a higher T_{core} for some cases in Periods 2 and 3, it was not statistically different from other groups before treatments started. These findings suggest that the use of baseline was not necessary. Thus, the differences observed between treatments described later may be attributed to treatment effects or due to individual animal differences, which could not be identified during the pre-treatment phase.

Period 2

In Period 2, both C-1 and C-.5T represented control groups. Since no differences ($P > 0.10$) existed between these two groups for any parameter during this period, C-1 and C-.5T were pooled and represented the control treatment (C) for Period 2.

Ambient conditions for Period 2. During Period 2 (Figure 2.6), the average daily values for maximum, mean and minimum T_a were 28.0, 23.2 and 18.5°C, respectively, while maximum and mean RH were 94.6 and 76.8%, respectively. Maximum T_a was greater than 28°C for 17 days and greater than 30°C for 4 days. Only for 3 days in this

Period, T_a did not reach 25°C. The highest T_a was seen on d 35 at 1500 h, reaching 32.6°C. Maximum RH was higher than 90% for 23 days in this period.

Performance parameters for Period 2. Feed intake was expressed on DM basis and DMI per unit of body weight (DMI per BW). As shown in Table 2.4, overall DMI and DMI per BW among treatments were not different (P > 0.10). Cvetkovic et al. (2005) also found no differences in DMI of dairy cows fed 56.7 g/d of Tasco vs. no Tasco, during heat stress. A trt x d interaction (P < 0.05; Figure 2.7a) revealed DMI of 0.25T was lower than C on d 20, 21, 25, and 32, and a trend (P < 0.10) was seen on d 26 and 33. Moreover, 0.25T had a lower (P < 0.05) DMI than 0.5T on d 19, with a trend (P < 0.10) on d 27 and 28. As shown in Figure 2.7b, a tendency for a trt x d interaction (P < 0.10) existed with 0.25T vs. 0.5T having lower DMI per BW on d 19 (P < 0.05) and 27 (P < 0.10), while 0.25T vs. C had lower (P < 0.05) DMI per BW on d 21, 25, 32, and 33. In general, 0.25T had lower DMI and DMI per BW than other treatments following a peak in T_a. Thus, the inclusion of 0.25% of Tasco to the diet reduce feed intake of dairy cows during some days in this period. Reduction in feed intake of animals consuming Tasco was also observed in other studies. Williams et al. (2009) found lower feed intake of steers fed a concentrate diet supplemented with 1% Tasco compared to controls, during early exposure to heat in climatic chambers, with no effects on final weight or ADG. Spiers et al. (2004a) observed a reduction in feed intake of steers fed an endophyte-infected tall fescue seed diet supplemented with 1% Tasco compared to 0 or 0.5% Tasco, after one week of heat exposure. This suggests that higher levels of Tasco supplementation may be deleterious for feed intake. This is in contrast to the current study, in which the lowest level of Tasco (0.25%) caused the major reduction in intake,

while 0.5% level vs. control did not differ (P > 0.10) at any time. Cattle reduce feed intake during heat stress periods as an attempt to reduce metabolic heat production (Hahn, 1999).

As shown in Table 2.4, there were no differences (P > 0.10) among treatments for milk production (Figure 2.7c), milk fat and protein yield or percent. Kellogg et al. (2006) did not find differences in milk production of cows exposed to heat stress and fed 0.25% of Tasco compared to control cows. In contrast, Cvetkovic et al. (2005) revealed an increase in milk production (from 33.5 to 35.2 kg/d) and milk protein yield (from 1.03 to 1.11 kg/d) for cows fed 56.7 g/d of Tasco compared to no Tasco. In the current study, a trend existed (P < 0.10) for a trt x time interaction for milk fat yield in Period 2 (Table 2.5), with 0.5T having a higher fat yield than C (P < 0.05) and 0.25T (P < 0.10) in the third week of the study (d 15 through 21).

For ECM and milk efficiency, no differences (P < 0.10) existed between treatments (Table 2.4). Supporting these findings, Cvetkovic et al. (2005) did not find differences in ECM for cows consuming 56.7 g/d of Tasco, compared to those not fed Tasco during summer heat stress. Moreover, in the current study, no differences (P > 0.10) were found for SCS between treatments. In contrast, Kellogg et al. (2006) found a lower somatic cell score for cows fed 0.25% Tasco, but only for one month during summer. In the current study, all treatments means for SCS were lower than a score of 4.0 (Table 2.4), meaning that, in average, the cows were healthy and did not have subclinical mastitis (score higher than 5.0). It is important to reinforce that all cows diagnosed with mastitis at some point during the experiment were removed from the data set, so it was expected that animals would not have SCS higher than 5.9 (750,000 cells).

The results for BW and BCS are presented as change, which represents the difference between the weight/condition from the beginning to the end of the period. No differences ($P > 0.10$) existed for BW and BCS change between treatments (Table 2.4).

Thermal status for Period 2: Respiration rate and skin temperatures.
Respiration rate and skin temperature results were expressed as an average per treatment for each time within day (0700, 1600 and 1900 h) when measurements were collected, as well as an average of those three measurements (Table 2.6). Moreover, the average per treatment for each day is shown in Figures 2.8a, b, and c, for RR, T_{ear} and T_{rump}, respectively. No differences existed ($P > 0.10$; Table 2.6 and Figure 2.8a) among treatments for RR, when expressed as the main effect or as interaction (trt x d, trt x h or trt x d x h). Cvetkovic et al. (2005) did not find any differences in RR for cows supplemented with 56.7 g/d of Tasco during summer. In contrast, Spiers et al. (2004a) found a temporarily reduction in RR of beef steers consuming an endophyte-infected tall fescue seed diet supplemented with 1% Tasco during heat stress compared to no Tasco. On the other hand, Williams et al. (2009) found a greater range of RR for steers fed 1% Tasco during heat exposure in climatic chambers; in the same study, steers fed 1% Tasco for 36 days at thermoneutrality and followed by heat exposure, had lower maximum RR and lower range of RR compared to the control treatment (Williams et al., 2009).

A trend for a trt x h interaction ($P < 0.10$; Table 2.6) existed for T_{ear} in Period 2, with 0.25T being lower than 0.5T at 1600 and 1900 h ($P < 0.05$ and 0.10, respectively). Moreover, at 1600 h, a tendency ($P < 0.10$) existed for C being higher than 0.25T. The average T_{rump} was lower ($P < 0.05$) for 0.25T as compared to C and 0.5T; moreover, T_{rump} for 0.25T was lower ($P < 0.01$) than C and 0.5T at 1600 and 1900 h (Table 2.6). Thus, the

54

inclusion 0.25% Tasco in the diet reduced the skin surface temperature of cows during this period. No benefits were seen with the higher level of inclusion of Tasco. It was speculated that this lower skin temperature may be related to the occasionally lower DMI of cows in this treatment, contributing to a reduced production of metabolic heat. In another study, however, supplementation of 56.7 g/d of Tasco to dairy cows during summer did not affect morning, afternoon, evening and average daily temperatures of rear udder skin (Cvetkovic et al., 2005). In the current study, the trt x d interaction was not significant ($P > 0.10$) for either T_{ear} (Figure 2.8b) or T_{rump} (Figure 2.8c).

Thermal status for Period 2: Core body temperature. No differences ($P > 0.10$) were observed among treatments for minimum, maximum or change in T_{core}, as well as for nighttime or daytime ranges (Table 2.6). Daily minimum and daytime average T_{core} of 0.5T was only numerically higher ($P = 0.12$ and $P = 0.11$, respectively) than other treatments. Differences are not expected in analysis performed for extended periods (i.e. 28 d), because of the great variation in T_{core} that occurs within days, preventing possible differences. In another study, no effects of Tasco were seen on rectal temperature of dairy cows supplemented with 56.7 g/d of Tasco for 5 weeks during summer heat stress (Cvetkovic et al., 2005). In contrast, lambs fed 2% of Tasco and submitted to stress of transportation in hot weather (temperature inside trailer ranged from 18.7 to 32.0ºC) had lower maximum and average ear canal temperature (Archer et al., 2007).

A trt x d interaction existed ($P < 0.05$) for nighttime and daytime, when comparing the daily average per treatment within each range of time. During the nighttime (Figure 2.9a), 0.5T had higher T_{core} than C for 9 days (d 14, 15, 17, 24, and 33 ($P < 0.05$); and d 19, 27, 31, and 34 ($P < 0.10$)), and higher T_{core} than 0.25T for 5 days (d

27 ($P < 0.05$); and d 9, 14, 16, and 25 ($P < 0.10$)). During the daytime (Figure 2.9b), T_{core}

of 0.5T was higher than C for 21 days (d 14, 15, 16, 18, 24, 28, 29, 30, 31, 32, 33, 34, and

35 ($P < 0.05$); and d 13, 17, 19, 20, 21, 22, 25, and 27 ($P < 0.10$)), and higher than 0.25T

for 10 days (d 14, 18, 21, 24, 27, 31, and 35 ($P < 0.05$); and d 20, 26, and 29 ($P < 0.10$)).

On d 15, a trend existed ($P < 0.10$) for 0.25T having higher T_{core} than C. Thus, for most

days in this period, the inclusion of 0.50% of Tasco in the diet caused T_{core} of dairy cows

to be increased. This was especially true during the daytime range, when 0.5T had higher

T_{core} than other treatments almost every day. In contrast, the T_{core} of C and 0.25T

treatments did not differ for most days, indicating that the lower level of Tasco did not

have the negative effects of the higher level (i.e. 0.50%). Also, even though the 0.25T

had lower skin temperature and occasionally lower DMI than other treatments during this

period, no effect was observed in T_{core} of cows.

　　　　Along with differences in T_{core}, some days also presented differences ($P < 0.10$) in

DMI. These days were 19, 20, 21, 25, 26, 27, 28, 32, and 33. However, no specific

pattern could be detected relating the differences in T_{core} with those in DMI; in other

words, treatment with higher T_{core} did not necessarily have higher or lower DMI. A

higher internal body temperature would be expected when the feed intake is elevated,

because of increased metabolic heat production. On the other hand, higher body

temperature as a cause of elevated ambient conditions, has been shown to reduce intake

of animals, in an attempt to reduce heat overload in the body (Kadzere et al., 2002).

　　　　A trt x h interaction existed ($P < 0.05$) during nighttime and daytime (Figure

2.10), when an hourly average of all days within the period was analyzed. The pattern of

T_{core} for all the treatments was similar throughout the day. However, 0.5T had about

0.2ºC higher T_{core} throughout the nighttime, and this difference increased to about 0.3ºC during most hours of the daytime. Minimum T_{core} of 0.5T treatment occurred at 0800 h, while for the other treatments, the minimum temperature happened later, at 1000 h. During the nighttime, 0.5T had higher T_{core} than C at 0500 h ($P < 0.10$) and at 0600 h ($P < 0.05$). During the daytime, 0.5T had higher T_{core} than C at 0700 h, and from 1000 to 1400 h ($P < 0.05$); at 0900 and 1500 h, 0.5T tended ($P < 0.10$) to be higher than C. Moreover, 0.5T had higher ($P < 0.05$) T_{core} than 0.25T from 1000 to 1300 h, with a tendency ($P < 0.10$) to be higher at 1500 and at 1600 h. No differences ($P > 0.10$) in T_{core} existed between C and 0.25T at any time. Thus, 0.5T had higher T_{core} than other treatments for almost all hours of the day.

Even though no significant differences ($P > 0.10$) were observed for milk production, there was some evidence that cows in the 0.5T had higher milk production than cows in the other groups (Figure 2.7c). This could have resulted in a higher internal body temperature of the 0.5T cows. High-producing dairy cows have usually higher feed intake and elevated metabolic rate and body heat production, resulting in elevated body temperature and higher vulnerability to heat stress (Kadzere et al., 2002). However, no differences ($P > 0.10$) in T_{core} existed between pre-assigned treatments. So, it was not possible to provide any specific reason why 0.5T had a higher T_{core} than other treatments. In contrast, rats fed a diet containing endophyte-infected tall fescue seed, supplemented with 1% Tasco, exhibited lower T_{core} when exposed to heat stress (Eichen et al., 2001; Spiers et al., 2004a). Moreover, steers grazing endophyte-infected tall fescue treated with Tasco-Forage (water soluble extract produced by alkaline hydrolysis and utilized as fertilizer for plants and soils) had lower rectal temperature compared to animals grazing

57

untreated forage during summer months (Saker et al., 2001); however, after animals were transported to feedlot facility, steers consuming Tasco had higher rectal temperature (Allen et al., 2001a).

Regression of body temperature as a function of ambient temperature for Period 2. The relationship between T_{rump}, T_{ear} and T_{core} with T_a are shown in Figure 2.11. Tasco had no influence ($P > 0.10$) on the slope for the relationships of T_{rump} (Figure 2.11a) or T_{ear} (Figure 2.11b) as functions of T_a, suggesting skin temperature of animals responded similarly to the increase in T_a, regardless of treatment. Two linear curves obtained from the quadratic regression of T_{core} vs. T_a during the daytime, Linear 1 and Linear 2, were tested for differences between treatment means and treatment slopes, respectively. For Linear 1 (representing thermoneutrality), no differences ($P > 0.10$) were found between treatments means. For Linear 2 (representing heat stress; Figure 2.11c), 0.25T had a lower ($P < 0.05$) slope than C for the relationship between T_{core} and T_a. No differences ($P > 0.10$) existed between other treatments. This indicates that the T_{core} of cows fed the 0.25T treatment increased less with the elevation in T_a compared to cows in the control treatment, maintaining a lower T_{core} during the day. Spiers et al. (2003) reported that steers grazing infected tall fescue, and previously fed 1% Tasco, had a reduced T_{core} of 0.3^{o}C, during first exposure to heat stress, compared to animals not fed Tasco, returning it to the level of animals on non-infected fescue. Moreover, Tasco delayed the initial increase of body temperature in response to ambient temperature for animals on the infected tall fescue, from 12 to 17°C (Spiers et al., 2003).

Period 3

Ambient conditions in Period 3. In Period 3 (Figure 2.12), the average daily
values for maximum, mean and minimum T_a were 31.0, 26.2 and 21.7°C, respectively,
while maximum and mean RH were 95.2 and 80.8%, respectively. The T_a exceeded 25°C
every day during this period, except on d 59 (max T_a = 24.4°C). According to Roenfeldt
(1998), the thermoneutral zone for lactating dairy cows ranges from 5 to 25°C, depending
on breed, size and level of production, and above this upper limit, cows need to spend
extra energy to dissipate heat and maintain normal body temperature. Maximum T_a was
greater than 28°C for 25 days, greater than 30°C for 20 days, and greater than 34°C for 3
days. The highest T_a was seen at 1500 h on d 49, reaching 34.9°C. Maximum RH was
higher than 90% for 23 days.

Performance parameters for Period 3. Treatments did not differ (P > 0.10; Table
2.7) in DMI (Figure 2.13a), DMI per BW (Figure 2.13b), milk production (Figure 2.13c),
milk components (fat and protein yield and percent), and SCS, for either main effects or
interaction trt x time. Body weight and BCS change were not different (P > 0.10)
between treatments (Table 2.7). No differences (P > 0.10) were found for the overall milk
efficiency (Table 2.7). However, there was a tendency (P < 0.10) for a trt x d interaction
(Figure 2.14), with 0.5T having a higher milk efficiency than C-1 on d 44, 49 and 56 (P <
0.10), higher than C-.5T on d 42 (P < 0.05), and d 49 and 60 (P < 0.10), and higher (P <
0.05) than all other groups on d 47. On d 56, 0.25T had higher (P < 0.05) milk efficiency
than all other treatments; 0.25T was higher (P < 0.10) than C-1 on d 57, 59 and 62, and
higher (P < 0.10) than C-.5T also on d 59. On d 62, C-.5T had higher efficiency than C-1
(P < 0.05) and 0.5T (P < 0.10). These results indicate that inclusion of Tasco increased

59

efficiency of milk production for some days during this period. No differences ($P > 0.10$)

on milk production or DMI were detected for this period, but when milk efficiency was

analyzed, differences were apparent, suggesting the analysis of milk efficiency may have

removed some of the variation among animals in a treatment, making the differences

more evident. Also, the highest peaks for milk efficiency for the Tasco treatments on d

42, 47 and 56 coincided with a low DMI for the corresponding day, suggesting that even

with a drop in DMI, milk production was not decreased, indicating a higher milk

efficiency for those days.

Thermal status for Period 3: Respiration rate and skin temperatures. Results for

RR and skin temperature are shown in Table 2.8. No differences ($P > 0.10$) were found

for RR, T_{ear} and T_{rump}, for overall average or for hours within day (0700, 1600 and 1900

h). Supporting these findings, supplementation of 56.7 g/d of Tasco during summer did

not affect morning, afternoon, evening and average daily temperatures of rear udder skin,

and respiration rates of dairy cows (Cvetkovic et al., 2005). The interaction trt x d was

not different ($P > 0.10$) for RR (Figure 2.15a) or T_{ear} (Figure 2.15b). For T_{rump}, however,

a trt x d interaction ($P < 0.05$; Figure 2.15c) existed with 0.25T having lower T_{rump} than

C-1 for 6 days (d 49, 55, 58, and 62 ($P < 0.05$); and d 41 and 47 ($P < 0.10$)) and lower

than C-.5T for 12 days (d 47, 48, 49, 55, 58, and 60 ($P < 0.05$); and d 44, 45, 50, 57, 62,

and 63 ($P < 0.10$)). The T_{rump} tended ($P < 0.10$) to be lower for 0.25T compared to 0.5T

for 3 days (d 53, 55, and 62), but higher for 1 day (d 39). Cows in the 0.5T treatment had

a lower T_{rump} than cows in C-1 for 4 days (d 39 and 58 ($P < 0.05$); and d 41 and 57 ($P <$

0.10) and lower than C-.5T for 3 days (d 47 and 57 ($P < 0.05$); and d 63 ($P < 0.10$)).

However, on d 53, 0.5T had higher ($P < 0.05$) T_{rump} than C-1 and C-.5T. The T_{rump} was

lower (P < 0.05) for C-.5T vs. C-1 on d 59, with a trend to be higher (P < 0.10) on d 63. The inclusion of 0.25% of Tasco in the diet reduced T_{rump} of cows on certain days; however, no effect was seen on ear skin temperature. In general, 0.25T had lower T_{rump} than other treatments on days with high T_a (above 30^oC). For Period 2, 0.25T had lower T_{rump} for the overall and hourly averages compared to other treatments. In Period 3, this lower T_{rump} of 0.25T was maintained, even though only the trt x d interaction was significant, suggesting that the effect of the 0.25% Tasco in lowering rump surface temperature lasted for most of the study. For the majority of the days, T_{rump} of C-1 and C-.5T did not differ, indicating that Tasco at 0.50% level, fed for a shorter length of time (28 days) did not affect rump surface temperature.

Thermal status for Period 3: Core body temperature. The results for T_{core} for daily minimum, maximum, and change (from maximum to minimum), and for the average of nighttime and daytime ranges are presented in Table 2.8. No differences (P > 0.10) in T_{core} existed among treatments for minimum, maximum, change or during nighttime and daytime. In contrast, beef steers fed 1% Tasco and housed in climatic chambers had lower range of rectal temperature during early exposure to heat (Williams et al., 2009). In the same study however, steers exposed to heat after consuming Tasco for 36 days at thermoneutrality had higher rectal temperature and greater range of rectal temperature, suggesting that Tasco fed for extended periods caused the opposite effect on body temperature of steers under heat stress (Williams et al., 2009).

A trt x d interaction existed (P < 0.05) for the nighttime (Figure 2.16a), when an average per treatment was compared. The 0.5T had higher T_{core} than C-1 for 12 days (d 39, 46, 52, and 53 (P < 0.05); and d 42, 44, 45, 47, 48, 54, 55, and 61 (P < 0.10)) and

higher than C-.5T for 6 days (d 36, 39, 55, and 61 ($P < 0.05$); and d 52 and 63 ($P < 0.10$)). Moreover, 0.5T had higher T_{core} than 0.25T for 6 days (d 36, 37, and 55 ($P < 0.05$); and d 39, 45, and 48 ($P < 0.10$)). A trend ($P < 0.10$) existed for lower T_{core} for C-1 compared to 0.25T on d 47; on d 53, C-1 was lower than 0.25T ($P < 0.05$) and C-.5T ($P < 0.10$). The trt x d interaction for the daytime range was not significant ($P > 0.10$; Figure 16b). As observed for Period 2, cows in the 0.5T treatment had elevated T_{core} compared to other treatments for most days in Period 3. In contrast, cows in the C-.5T treatment, which also received 0.50% of Tasco during this period, did not exhibit elevated T_{core}. The T_{core} of C-1, C-.5T and 0.25T treatments did not differ for most days, indicating that the low level of Tasco (i.e. 0.25%) and the high level of Tasco (i.e. 0.50%) fed for a shorter period of time (i.e. 28 vs. 56 days) did not affect core temperature of dairy cows. Also, despite the lower T_{rump} of cows in the 0.25T treatment on certain days in this period, core temperature was not affected, maintaining the same level as C-1 and C-.5T.

In Period 3, differences ($P < 0.10$) in T_{core} and T_{rump} existed in corresponding days (d 39, 44, 45, 47, 48, 53, 55, and 63). However, there was not a specific pattern for T_{core} differences that could be related to the differences in T_{rump}, therefore, treatment with higher T_{core} did not necessarily have higher or lower T_{rump}. Even though T_{core} of 0.5T was higher for most of the days compared to other treatments, T_{rump} of cows in this treatment was lower only for certain days, compared to C-1 and C-.5T. Moreover, RR of 0.5T was not different from other treatments, suggesting that cows in the 0.50% Tasco did increase respiratory heat loss, as an attempt to lose heat and counteract the higher T_{core}. So, neither respiratory or cutaneous heat loss were increased in order to enhance heat dissipation and

avoid an increase in core temperature, suggesting that cows in the 0.50% Tasco may have naturally higher T_{core}.

A trend ($P < 0.10$) existed for a trt x h interaction for nighttime and daytime (Figure 2.17), for the hourly average of all days within the period. The pattern of T_{core} for all the treatments was similar throughout the day, with 0.5T being 0.2 to 0.3°C fold higher than other treatments for most part of the day. Minimum T_{core} for all treatments occurred at 0800 h. During the nighttime, 0.5T tended ($P < 0.10$) to have a higher T_{core} than C-1 for every hour from 0300 to 0600 h. The 0.5T also tended ($P < 0.10$) to have higher T_{core} than C-.5T at 0100, 0300 and 0400 h, and higher than 0.25T at 0300 h. During the daytime, 0.5T had a higher T_{core} than C-1 at 1000 h ($P < 0.05$), 1100 and 1600 h ($P < 0.10$), higher than C-.5T at 1100 h ($P < 0.05$), 1000 and 1200 h ($P < 0.10$), and higher than 0.25T at 1200 and 1600 h ($P < 0.10$). No differences ($P > 0.10$) in T_{core} were found between the other groups at any time, indicating that the 0.50% of Tasco fed for 56 days (i.e. 0.5T) elevated core temperature of dairy cows. Moreover, 0.50% of Tasco fed for 28 days (i.e. C-.5T) did not affect T_{core} of cows, which was maintained at the same level of control cows.

From the analysis of T_{core} for Periods 2 and 3, it was noted that cows in the 0.5T treatment had consistent higher T_{core} compared to other groups. Even though no differences were found for pre-assigned treatments, there is some evidence that cows in this treatment had an elevated core temperature throughout the whole study. This suggests that the higher T_{core} of 0.5T compared to other groups may be attributed to animal individual differences, and not treatment effects. Possibly, cows assigned to the 0.5T had a natural higher core temperature at thermoneutrality, what was carried out

throughout the study. This is supported by the fact that cows in this treatment, despite of the higher T_{core}, did not exhibit higher RR or skin temperatures, so they did not attempt to dissipate body heat. Moreover, cows in the C-.5T, which received the same level of Tasco (0.50%) starting on the first day of Period 3, did not have elevated T_{core}, supporting the idea that differences seen for core temperature of cows in 0.5T were likely to be animal individual differences rather than a Tasco effect. Thus, further research is necessary in order to identify true effects of the inclusion of 0.50% of Tasco on T_{core} of dairy cows.

Some of the days with differences in T_{core} ($P < 0.05$ or $P < 0.10$) had a high T_a, with peak temperature higher than 30ºC, certainly presenting heat stress conditions and possibly causing heat stress to cows. These days were 36, 43, 44, 49, 61, and 63. However, even when the T_a was the highest, few differences between treatments could be observed. Furthermore, even the hottest day in the study did not have a T_a higher than 35°C, which cannot be considered an extreme condition, even though above the upper critical limit for dairy cows (Roenfeldt, 1998). The few differences observed between treatments could be related to the lack of extreme ambient conditions during the study. In a previous study, Tasco had no benefits on the immune system of steers grazing endophyte-free pasture treated with Tasco during the grazing period (Saker et al., 2001), but the effect was reversed after animals were exposed to the stress of transportation to the feedlot (Allen et al., 2001a). The authors suggested that Tasco effect on immune function is dependent on the presence of a stressor. This could explain the little benefit of Tasco supplementation in the current study, since no extreme hot days or prolonged heat stress situations were encountered.

Regression of body temperature as a function of ambient temperature for Period 3. The effects of Tasco on body temperature relative to the increase in T_a during the day were evaluated. Linear regression of T_{rump} vs. T_a (Figure 2.18a) demonstrated that 0.25T had lower ($P < 0.05$) slope than all other groups. For T_{ear} vs. T_a (Figure 2.18b), a trend ($P < 0.10$) existed for 0.25T having lower slope compared to C-1 and C-.5T. For T_{core} vs. T_a during the daytime, no differences ($P > 0.10$) existed between treatment means for Linear 1, which represented thermoneutrality. For T_{core} vs. T_a at heat stress (Linear 2; Figure 2.18c), 0.25T and 0.5T had lower ($P < 0.05$) slopes than C-1 and C-.5T; no differences ($P > 0.10$) were found between 0.25T and 0.5T or between C-1 and C-.5T. Thus, even though 0.5T had a higher T_{core} throughout the study compared to other treatments, for Period 3, the group had less of an increase in T_{core} compared to control groups, as T_a increased during the day. The results of lower slope of 0.25T for the increase in T_{rump}, T_{ear} and T_{core} as functions of T_a indicates that cows fed 0.25% Tasco were better able to maintain a lower body temperature with the increase in T_a during the day. In work done by Spiers and co-authors (2004a), Tasco supplementation at 1% level to steers fed an endophyte-infected tall fescue seed diet reduced hyperthermia caused by fescue toxicosis. The steers also had reduced feed intake, suggesting that the reduced energy intake may have reduced metabolic heat production and body temperature (Spiers et al., 2004a). In the current study, a lower dose of Tasco (0.25%) was observed to cause similar effects of reduced body temperature and feed intake of dairy cows.

It was also noted that different responses were obtained for the relationship of body temperature and T_a during Periods 2 and 3. In Period 2, 0.25T had a lower slope compared to other treatment only for the relationship between T_{core} and T_a, while no

differences existed for T_{rump} vs. T_a and T_{ear} vs. T_a. On the other hand, in Period 3, 0.25T had lower slope for all T_{rump}, T_{ear} and T_{core} vs. T_a, and, in addition, 0.5T had lower slope of T_{core} vs. T_a, compared to the C-1 and C-.5T. It was speculated that this variation in the response may be attributed to greater ambient temperature in Period 3 compared to Period 2, which may have caused a higher level of heat strain to cows.

CONCLUSIONS

From this study, the inclusion of 0.25% Tasco in the total mixed ration reduced dry matter intake of cows on certain days in Period 2; however, the reduction in dry matter intake did not occur in Period 3. Even though dry matter intake was lowered at certain times in Period 2, milk production was not different among treatments. The major effect of 0.25% Tasco was the lower increase in skin and core temperatures relative to the increase in ambient temperature in Period 3, when more hot conditions were encountered. However, no positive effect was seen on the overall internal body temperature of cows in this treatment. Moreover, the increase of Tasco concentration in the diet, from 0.25% to 0.50%, did not present any benefit on body surface temperature, and in contrast, there was some evidence that this increase may elevate core body temperature, even though it is speculated that some of the differences observed may be due to animal's individual differences. The inclusion of Tasco at 0.50% level in the feed for 28 days during Period 3 did not have any effect on dry matter intake, milk production, respiration rate, body core or surface temperatures.

Table 2.1. Composition of the total mixed ration

Feed ingredient	% of DM
Alfalfa hay	13.8
Alfalfa silage	13.8
Corn silage	18.1
Shelled corn	20.74
Soybean meal, 48%	3.8
Whole cottonseed	4.8
Roasted soybeans	4.0
Wet brewer's grains	7.2
Soybean hulls	11.7
Dicalcium phosphate	0.51
Limestone	0.62
Sodium bicarbonate	0.44
TM salt	0.26
Vitamin ADE premix #1	0.09
Vitamin E premix	0.09
RTM trace mineral	0.05
Chemical Analysis	
Dry matter	52.1
Crude protein	18.1
NDF	42.2
ADF	26.8

Figure 2.1. Average core body temperature (T$_{core}$) of all animals for the whole study throughout the nighttime, daytime, and both milking and feeding periods
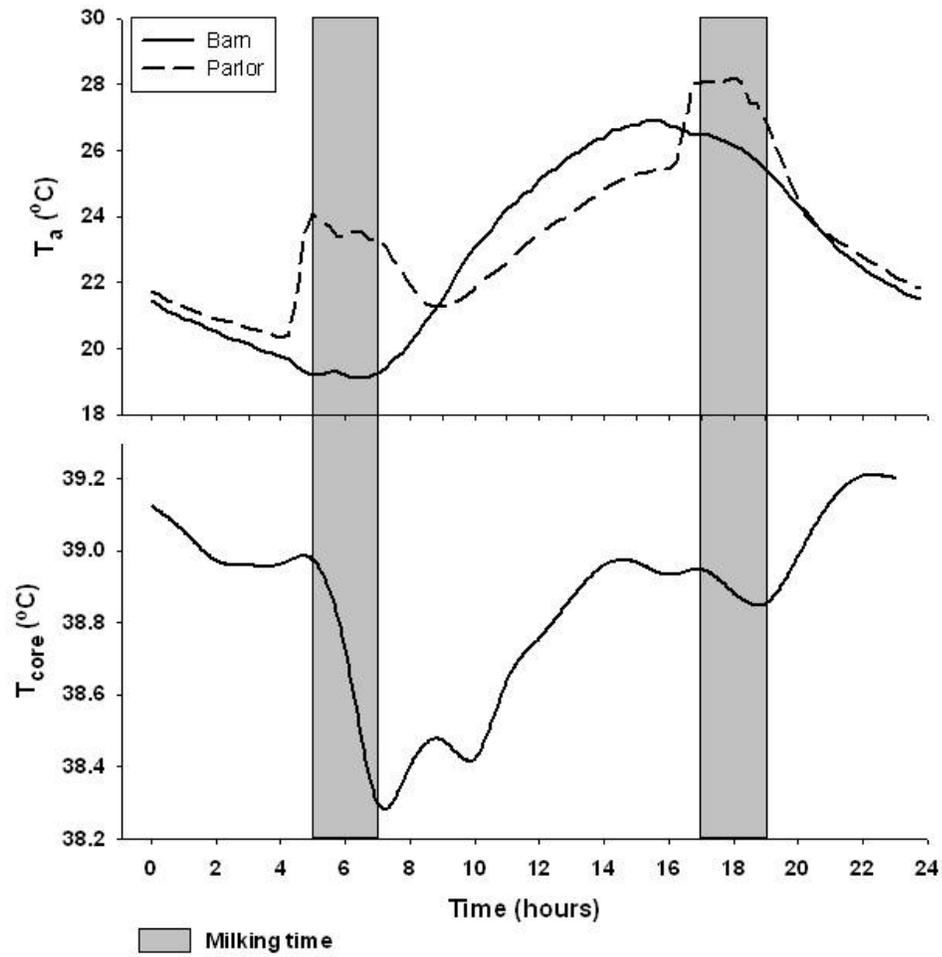
Figure 2.2. Comparison of average ambient temperature (T_a) in the barn and milking parlor during summer 2009, relative to average core body temperature (T_{core}) during summer 2008
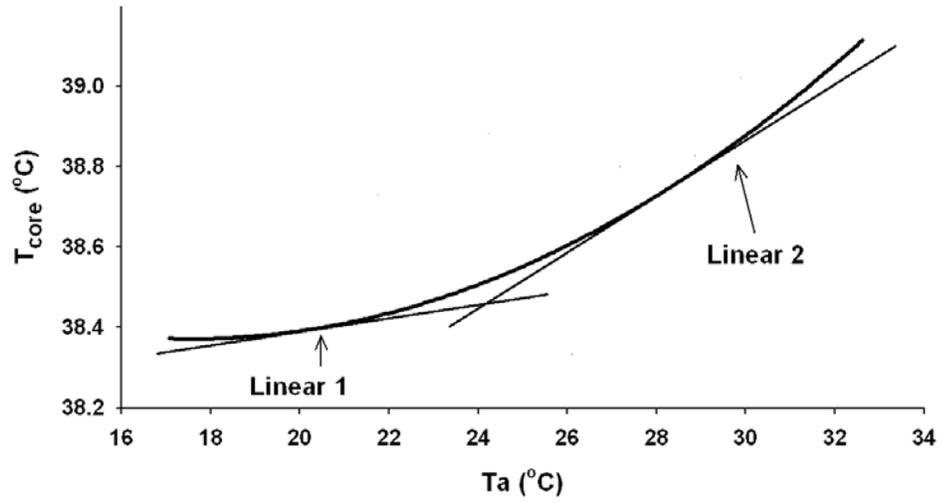
Figure 2.3. Example of quadratic regression for core body temperature (T_{core}) as a function of ambient temperature (T_a), showing two linear curves representing thermoneutrality (Linear 1) and heat stress (Linear 2) regions

70

June 2	June 9		July 7	August 3
Period 1 (7 d)	**Period 2** (28 d)		**Period 3** (28 d)	

Adjustment period

Treatments:
- C-1 (control)
- C-.5T (control)
- 0.25T (0.25% Tasco)
- 0.5T (0.50% Tasco)

Treatments:
- C-1 (control)
- C-.5T (0.50% Tasco)
- 0.25T (0.25% Tasco)
- 0.5T (0.50% Tasco)

- Feed Intake
- Milk production and composition
- Respiration rate
- Ear and rump skin temperatures
- Core body temperature
- Body weight and BCS
- Ambient temperature and relative humidity

Figure 2.4. Description of the study.
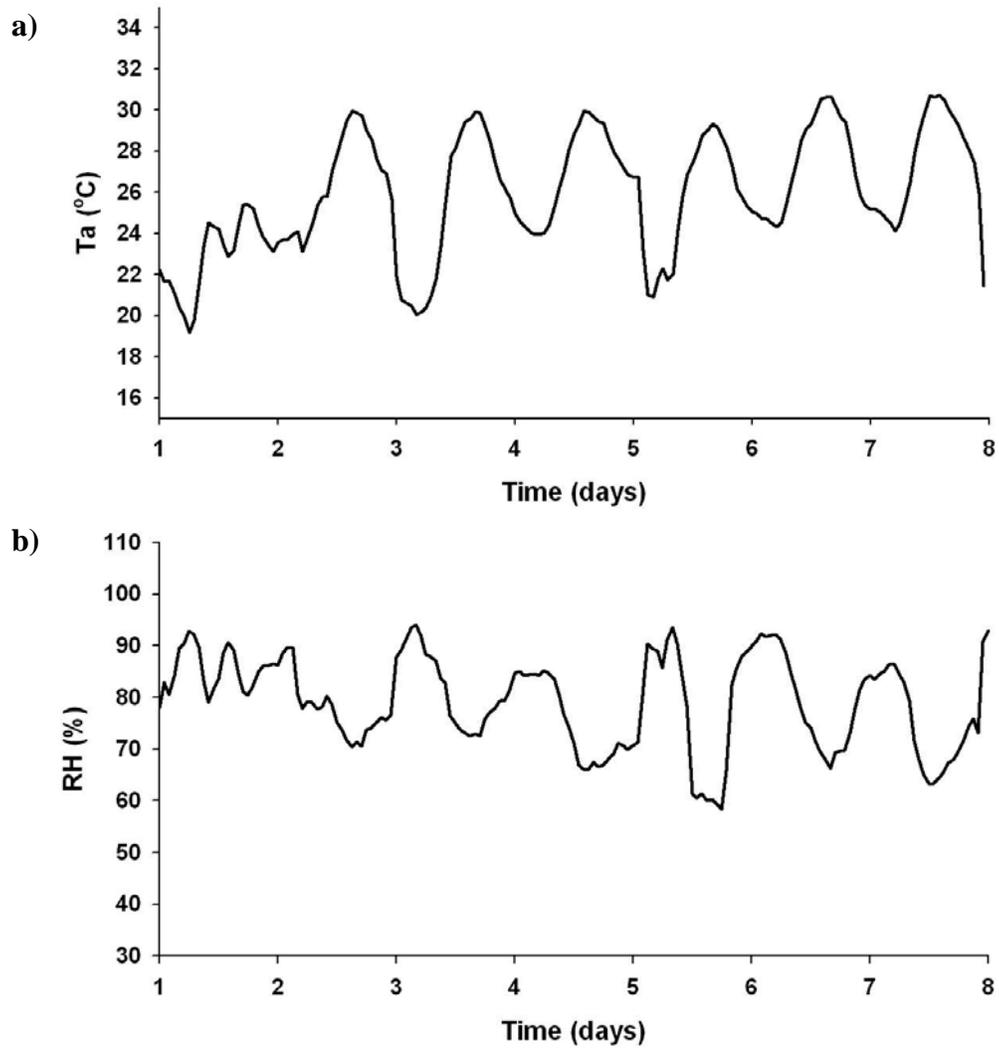
Figure 2.5. Ambient conditions within the barn during Period 1. They include: a) ambient temperature (T_a); b) percent relative humidity (RH).

Table 2.2. Influence of Tasco on performance parameters during Period 1: DMI, milk production and composition, milk efficiency, body weight, and BCS

Item	Treatment				SE_{pooled}	P value
	C-1	C-.5T	0.25T	0.5T		
Cows, n	8	8	8	8		
DMI, kg/d	26.56	25.08	24.70	24.66	1.07	0.56
DMI per BW, kg/kg	0.042	0.037	0.040	0.040	0.0017	0.26
Milk, kg/d	33.00	32.15	32.81	33.94	1.93	0.93
Milk components						
Fat, %	3.29	3.55	3.38	3.32	0.19	0.78
Fat yield, kg/d	1.05	1.17	1.13	1.12	0.06	0.58
Protein, %	2.82	2.68	2.77	2.89	0.10	0.46
Protein yield, kg/d	0.90	0.90	0.94	0.97	0.05	0.60
ECM[1], kg/d	31.18	30.70	31.33	32.50	1.56	0.84
ECM/DMI[2], kg/kg	1.19	1.24	1.29	1.33	0.05	0.11
SCS[3]	3.10	2.62	2.85	1.89	0.85	0.66
BW change, kg	1.0	7.8	1.9	3.1	5.4	0.83
BCS change	-0.03	0.04	-0.09	-0.13	0.09	0.63

[1] ECM = energy-corrected milk = (0.327 x milk yield [kg/d]) + (12.95 x fat yield [kg/d]) + (7.2 x protein yield [kg/d]);
[2] ECM/DMI = milk efficiency;
[3] SCS = SCC linear score = $\log_2$ (SCC/100,000) + 3.

Table 2.3. Influence of Tasco on thermal status during Period 1: respiration rate (RR), ear (T_{ear}) and rump skin temperatures (T_{rump}) in the morning, afternoon, evening, and daily averages, and core body temperature (T_{core}) for daily minimum, maximum, and change, and average for nighttime and daytime ranges

Item	Treatment				SE_{pooled}	P value
	C-1	C-.5T	0.25T	0.5T		
RR, bpm						
0700 h	47.65	45.47	44.20	45.50	3.34	
1600 h	66.50	69.04	64.75	68.75	3.34	0.21
1900 h	64.75	60.43	64.60	64.05	3.34	
Daily average	59.63	58.31	57.85	59.43	3.04	0.96
T_{ear}, °C						
0700 h	33.94	33.86	34.09	34.20	0.21	
1600 h	34.91	35.14	35.17	35.31	0.21	0.97
1900 h	35.24	35.32	35.27	35.57	0.21	
Daily average	34.70	34.77	34.84	35.03	0.15	0.29
T_{rump}, °C						
0700 h	33.80	34.22	34.06	34.08	0.21	
1600 h	35.53	35.36	35.22	35.43	0.21	0.49
1900 h	35.41	35.21	35.02	35.34	0.21	
Daily average	34.91	34.93	34.77	34.95	0.15	0.82
T_{core}, °C						
Entire day						
Min	38.07	38.13	38.14	38.38	0.11	0.25
Max	39.87	39.83	39.83	39.97	0.14	0.87
Change[1]	1.80	1.70	1.69	1.59	0.09	0.48
Nighttime[2]	39.15	39.26	39.26	39.33	0.13	0.82
Daytime[3]	38.90	39.00	38.97	39.16	0.12	0.50

[1] Change = maximum – minimum;
[2] Range of hours from 0 to 0600 h;
[3] Range of hours from 0700 to 1600 h.
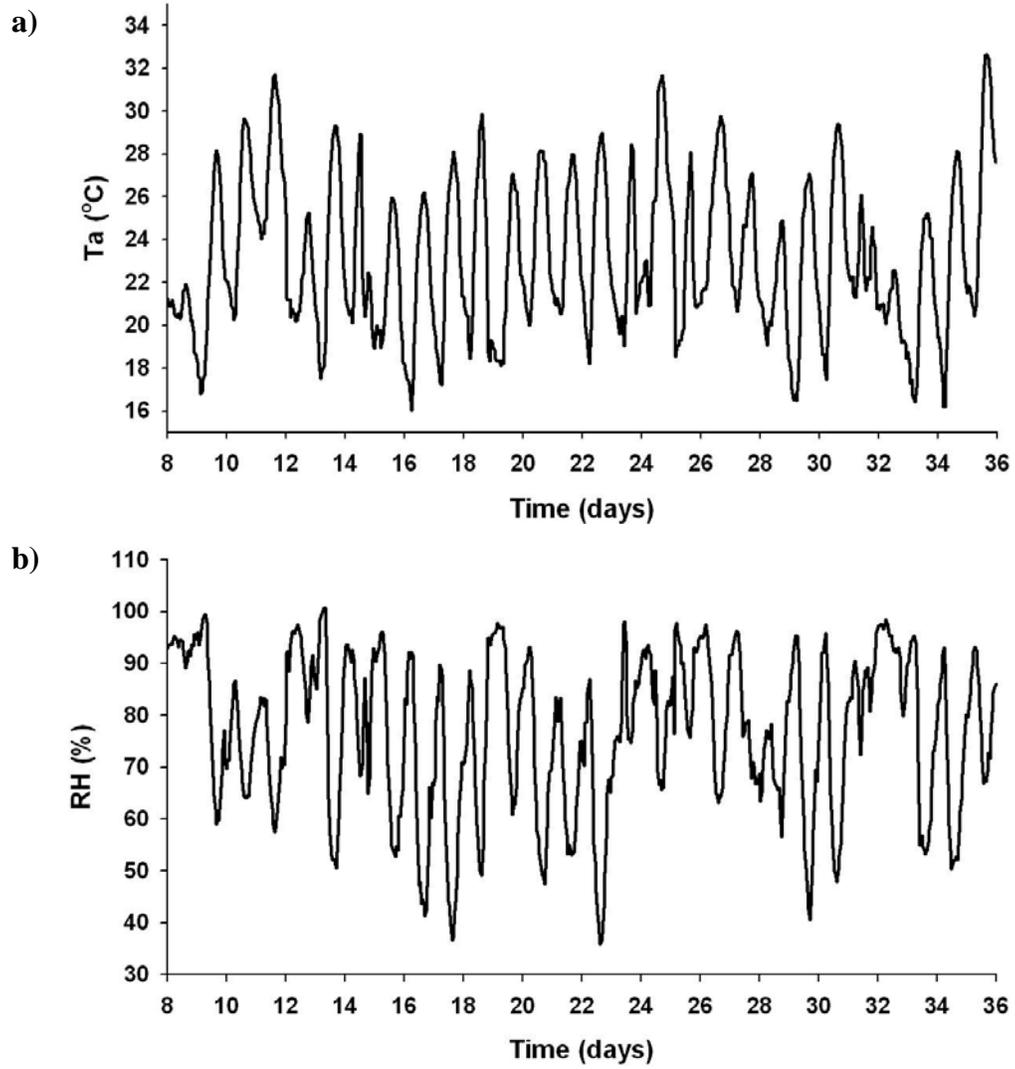
Figure 2.6. Ambient conditions within the barn during Period 2. They include: a) ambient temperature (T_a); b) percent relative humidity (RH).
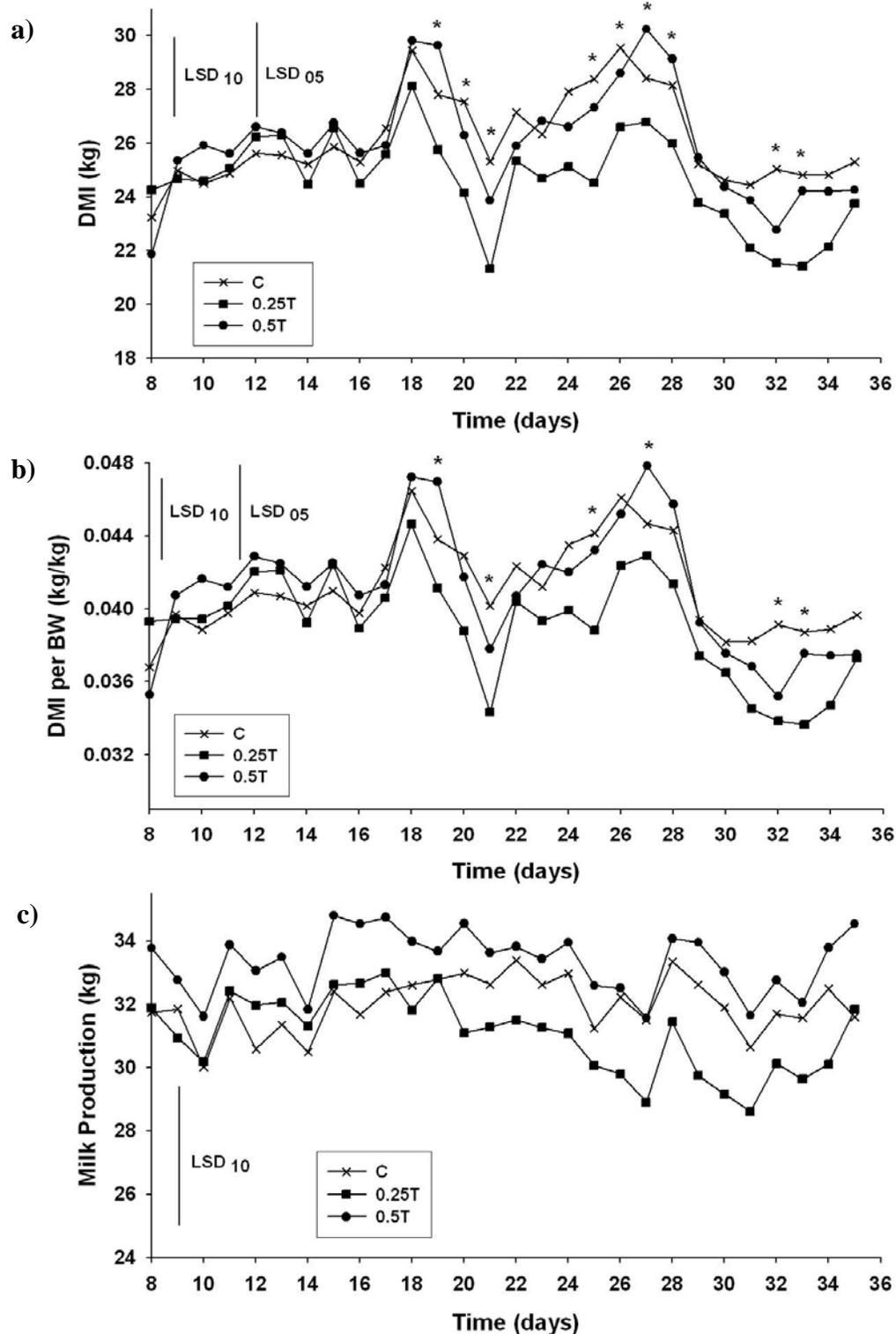
Figure 2.7. Influence of Tasco on daily average of performance parameters during Period 2. They include: a) DMI; b) DMI per BW; c) milk production. (*) Days with differences at 5 or 10% between one or more treatments; see text for details. For Period 2, C-1 and C-.5T have been combined and represent the control group (C).
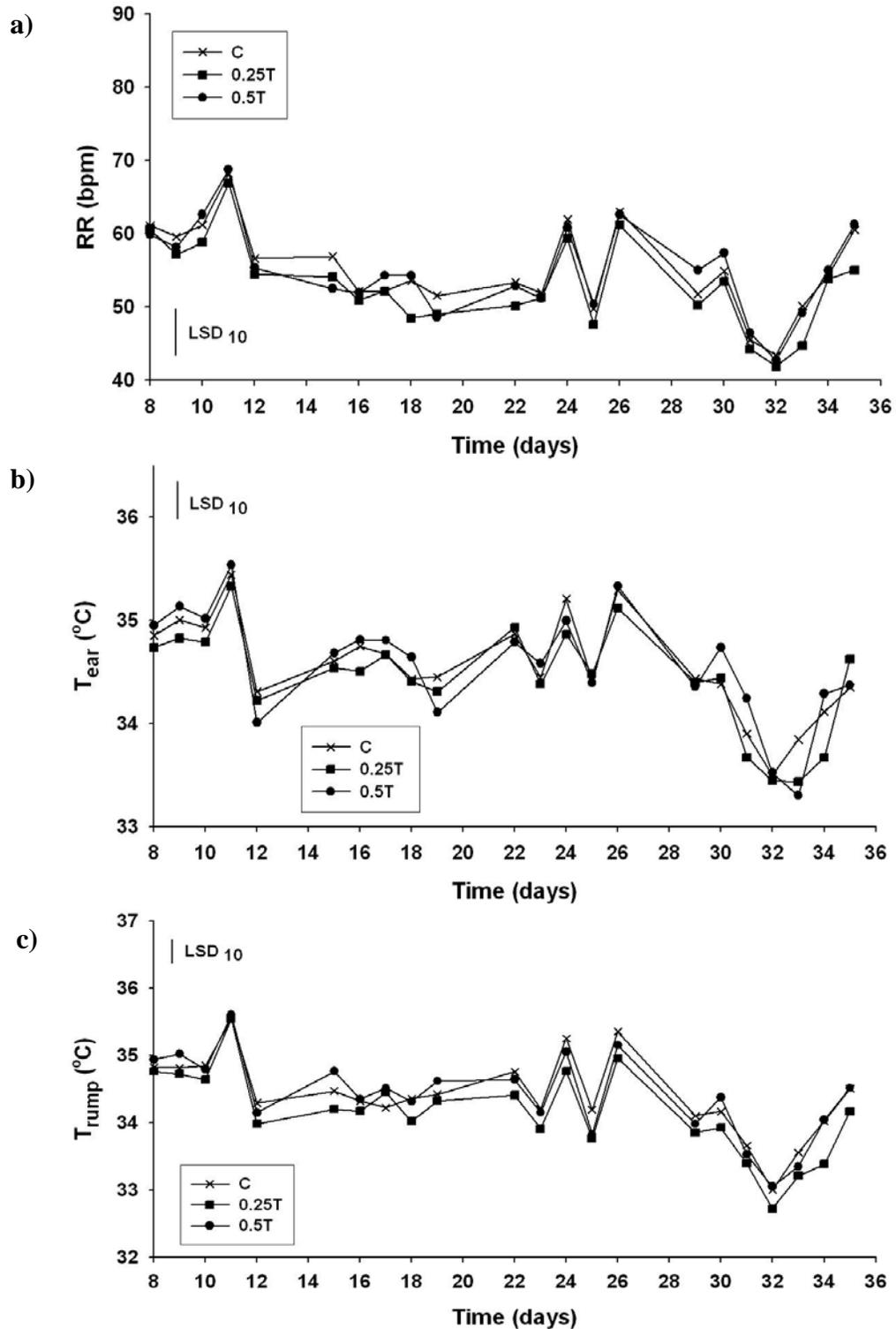
Figure 2.8. Influence of Tasco on daily average of thermal status during Period 2. They include: a) respiration rate (RR); b) ear skin temperature (T_{ear}); c) rump skin temperature (T_{rump}). For Period 2, C-1 and C-.5T have been combined and represent the control group (C).
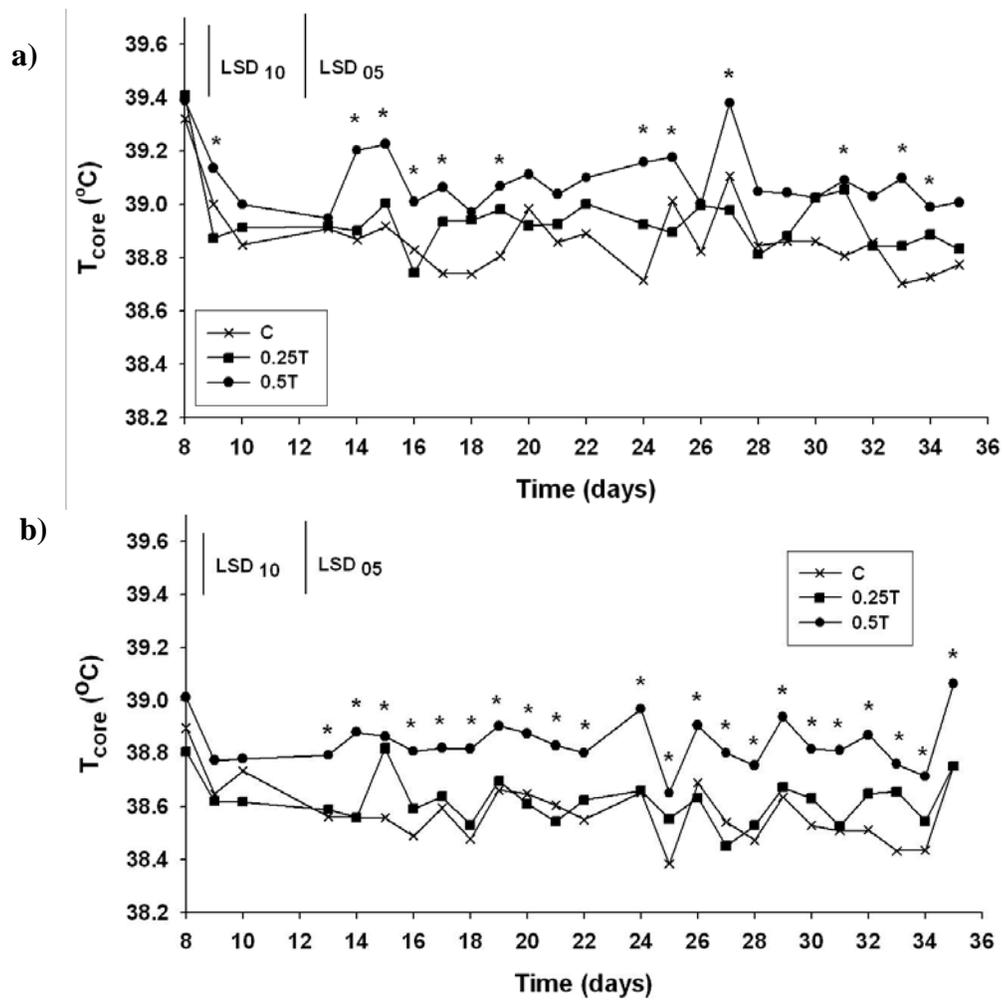
Figure 2.9. Influence of Tasco on daily average core body temperature (T_{core}) during Period 2. They include: a) average T_{core} during the nighttime range; b) average T_{core} during the daytime range. (*) Days with differences at 5 or 10% between one or more treatments; see text for details. For Period 2, C-1 and C-.5T have been combined and represent the control group (C).

Table 2.4. Influence of Tasco on performance parameters during Period 2: DMI, milk production and composition, milk efficiency, body weight, and BCS

Item	Treatment			SE$_{pooled}$	P value
	C[1]	0.25T	0.5T		
Cows, n	16	8	8		
DMI, kg/d	26.14	24.60	26.03	1.02	0.50
DMI per BW, kg/kg	0.040	0.039	0.041	0.0014	0.51
Milk, kg/d	31.98	31.04	33.35	1.61	0.60
Milk components					
Fat, %	3.47	3.48	3.42	0.14	0.93
Fat yield, kg/d	1.11	1.09	1.15	0.05	0.73
Protein, %	2.90	2.89	2.97	0.07	0.57
Protein yield, kg/d	0.92	0.92	0.99	0.04	0.45
ECM[2], kg/d	31.43	30.24	32.84	1.38	0.39
ECM/DMI[3], kg/kg	1.22	1.24	1.28	0.04	0.62
SCS[4]	3.35	2.97	2.53	0.71	0.72
BW change, kg	8.7	12.6	26.9	8.0	0.27
BCS change	-0.10	0.03	0.06	0.10	0.40

[1] For Period 2, C-1 and C-.5T have been combined and represent the control group (C);
[2] ECM = energy-corrected milk = (0.327 x milk yield [kg/d]) + (12.95 x fat yield [kg/d]) + (7.2 x protein yield [kg/d]);
[3] ECM/DMI = milk efficiency;
[4] SCS = SCC linear score = $\log_2$ (SCC/100,000) + 3.

Table 2.5. Influence of Tasco on fat yield (kg/d) for each week of Period 2[1]

Week	Treatment			SE$_{pooled}$	P value
	C[1]	0.25T	0.5T		
Week 2	1.07	1.13	1.15	0.06	
Week 3[2]	1.06 [b]	1.07 [ab]	1.23 [a]	0.07	
Week 4	1.17	1.10	1.18	0.07	0.08
Week 5	1.12	1.07	1.03	0.07	

[a,b] Means with different superscripts in column differ (P < 0.05);
[1] For Period 2, C-1 and C-.5T have been combined and represent the control group (C);
[2] There may be differences at other levels than that showed in table; see text for details.

Table 2.6. Influence of Tasco on thermal status during Period 2: respiration rate (RR), ear (T_{ear}) and rump skin temperatures (T_{rump}) in the morning, afternoon, evening, and daily averages, and core body temperature (T_{core}) for daily minimum, maximum, and change, and average for nighttime and daytime ranges

Item	Treatment			SE_{pooled}	P value
	C[1]	0.25T	0.5T		
RR, bpm					
0700 h	50.79	48.91	50.58	2.43	
1600 h	58.93	56.67	59.18	2.43	0.98
1900 h	55.71	53.31	55.32	2.43	
Daily average	55.14	52.96	55.03	2.37	0.69
T_{ear}, °C					
0700 h	34.16	34.12	34.06	0.10	
1600 h[2]	34.88 ab	34.71 b	34.94 a	0.10	0.09
1900 h	34.62 AB	34.51 B	34.71 A	0.10	
Daily average	34.55	34.44	34.57	0.09	0.27
T_{rump}, °C					
0700 h	33.82	33.68	33.67	0.10	
1600 h	34.86 c	34.54 d	34.88 c	0.10	0.004
1900 h	34.54 c	34.23 d	34.63 c	0.10	
Daily average	34.40 a	34.15 b	34.39 a	0.09	0.05
T_{core}, °C					
Entire day					
Min	37.76	37.73	38.06	0.11	0.12
Max	39.45	39.48	39.65	0.10	0.30
Change[3]	1.69	1.74	1.60	0.06	0.22
Nighttime[4]	38.87	38.94	39.09	0.10	0.29
Daytime[5]	38.58	38.61	38.84	0.09	0.11

[a,b] Means with different superscripts in row differ (P < 0.05);
[A,B] Means with different superscripts in row differ (P < 0.10);
[c,d] Means with different superscripts in row differ (P < 0.01);
[1] For Period 2, C-1 and C-.5T have been combined and represent the control group (C);
[2] There may be differences at other levels than that showed in table; see text for details;
[3] Change = max – min;
[4] Range of hours from 0 to 0600 h;
[5] Range of hours from 0700 to 1600 h.
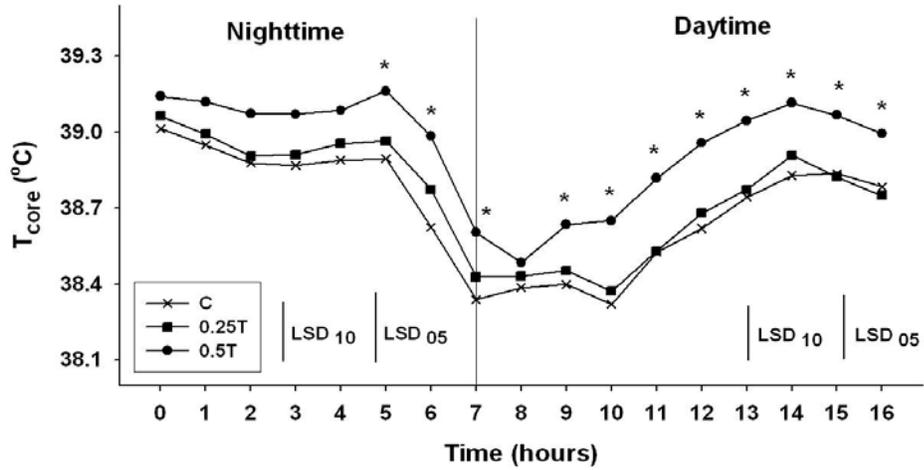
Figure 2.10. Influence of Tasco on the hourly average core body temperature (T_core) during nighttime and daytime for Period 2. (*) Hours with differences at 5 or 10% between one or more treatments; see text for details. For Period 2, C-1 and C-.5T have been combined and represent the control group (C).
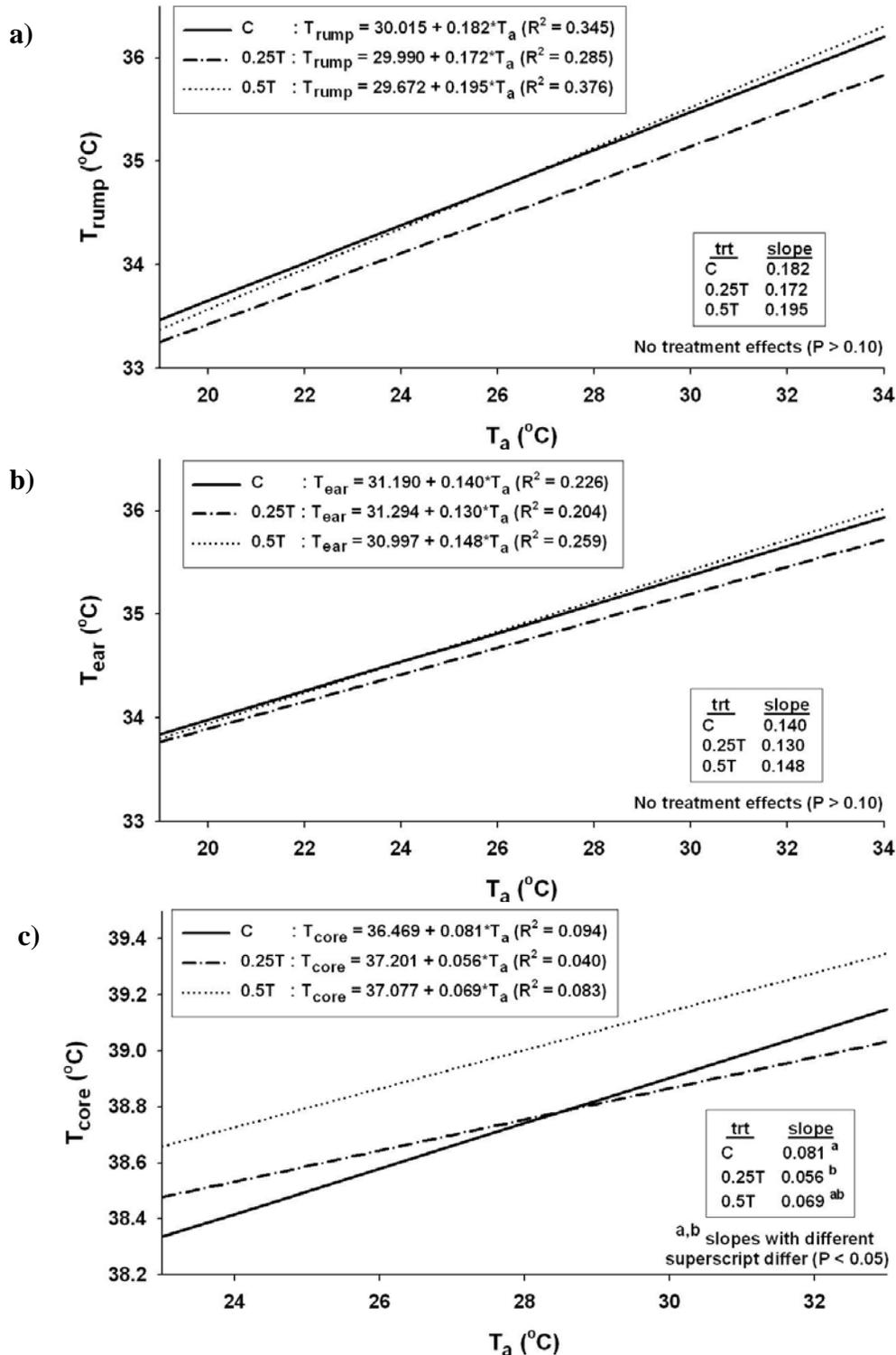
Figure 2.11. Influence of Tasco on the change of thermal status as functions of ambient temperature (T_a) during Period 2. They include: a) rump skin temperature (T_{rump}) vs. T_a; b) ear skin temperature (T_{ear}) vs. T_a; c) core body temperature (T_{core}) during the daytime vs. T_a. For Period 2, C-1 and C-.5T have been combined and represent the control group (C).
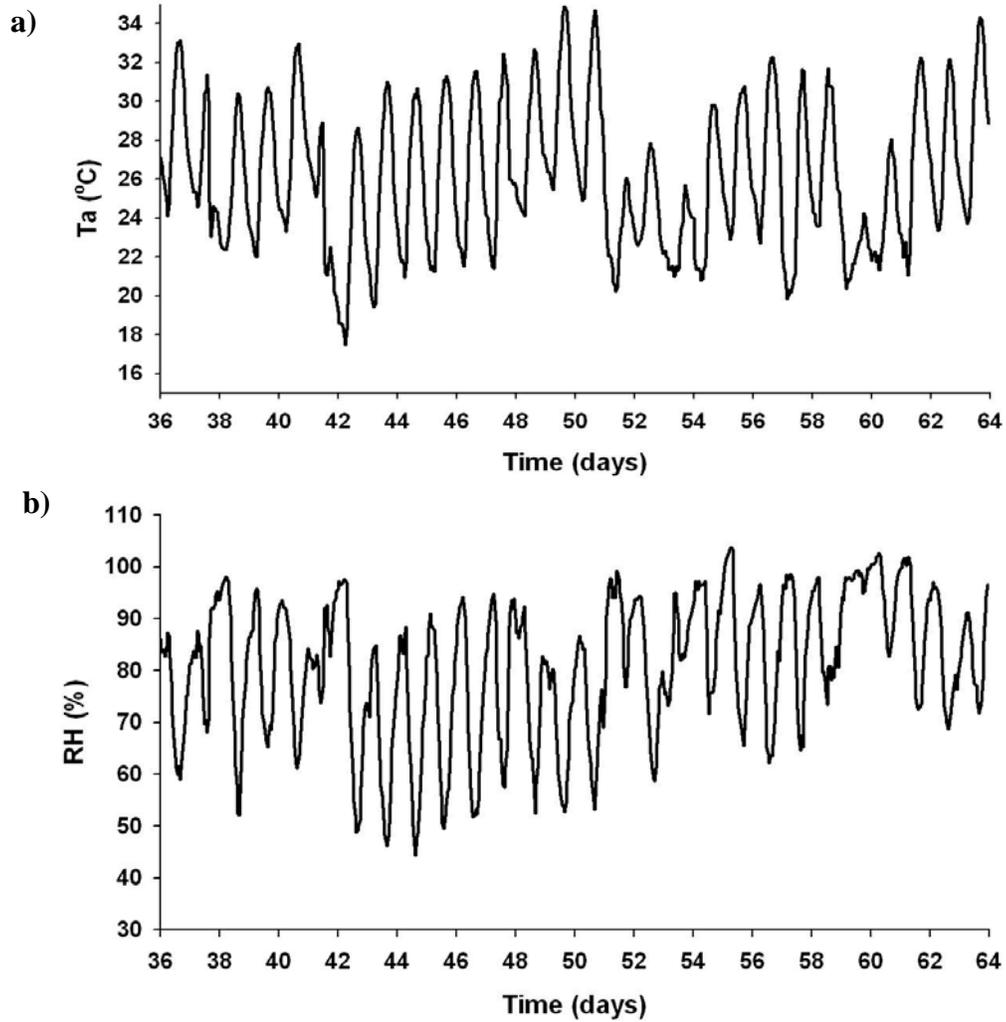
Figure 2.12. Ambient conditions within the barn during Period 3. They include: a) ambient temperature (T_a); b) percent relative humidity (RH).

84
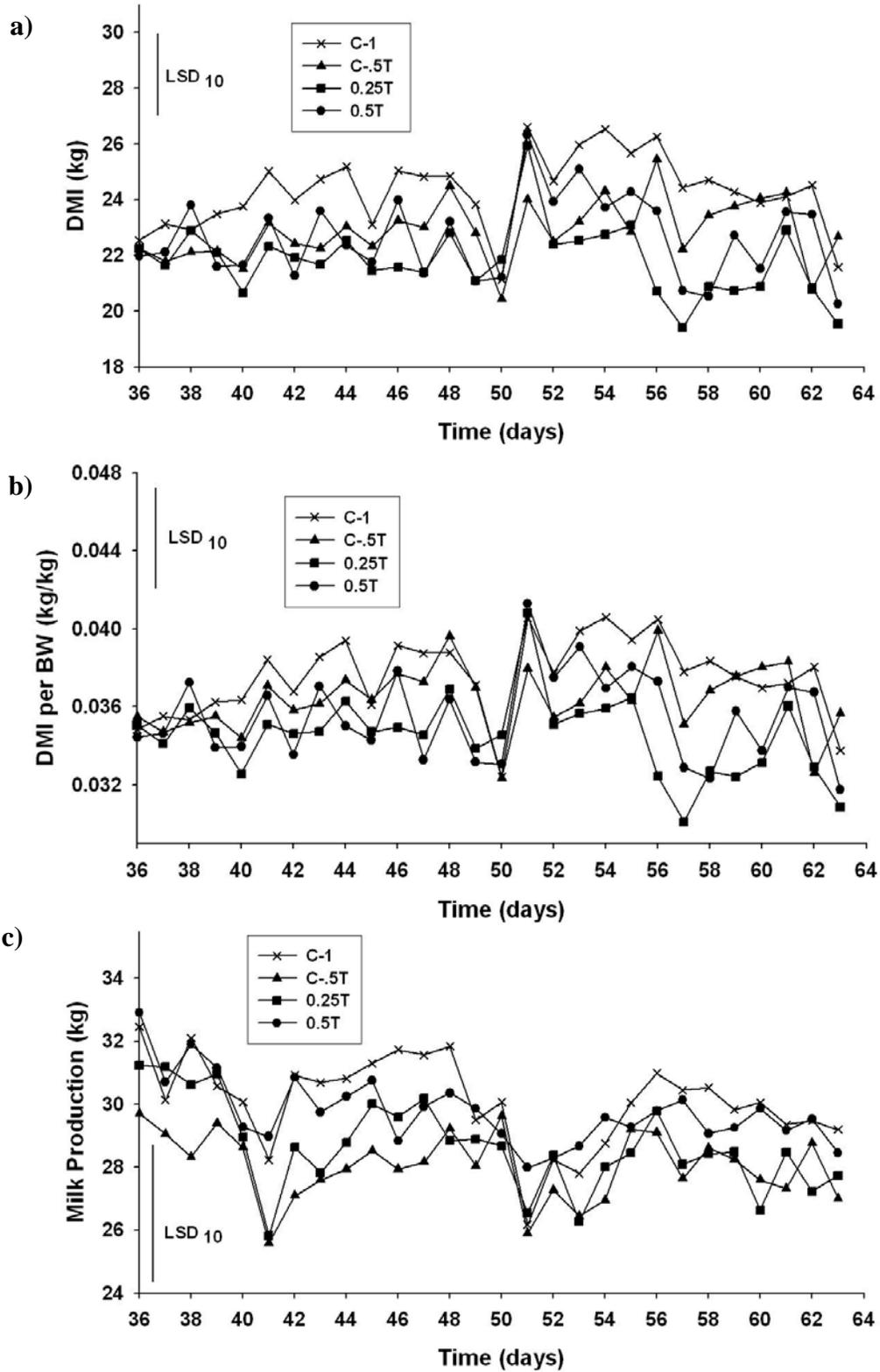
Figure 2.13. Influence of Tasco on daily average of performance parameters during Period 3. They include: a) DMI; b) DMI per BW; c) milk production.
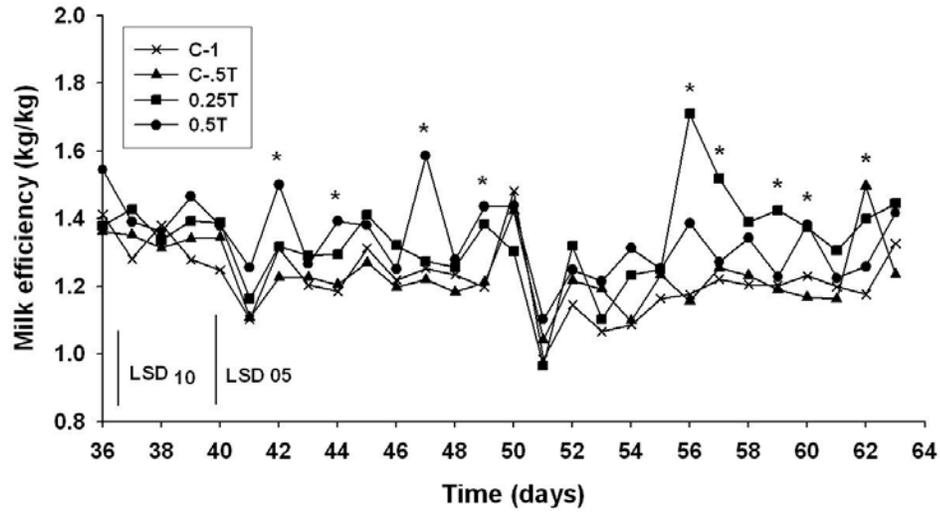
Figure 2.14. Influence of Tasco on milk efficiency (energy-corrected milk per DMI) for Period 3. Energy-corrected milk = (0.327 x milk yield [kg/d]) + (12.95 x fat yield [kg/d]) + (7.2 x protein yield [kg/d]). (*) Days with differences at 5 or 10% between one or more treatments; see text for details.
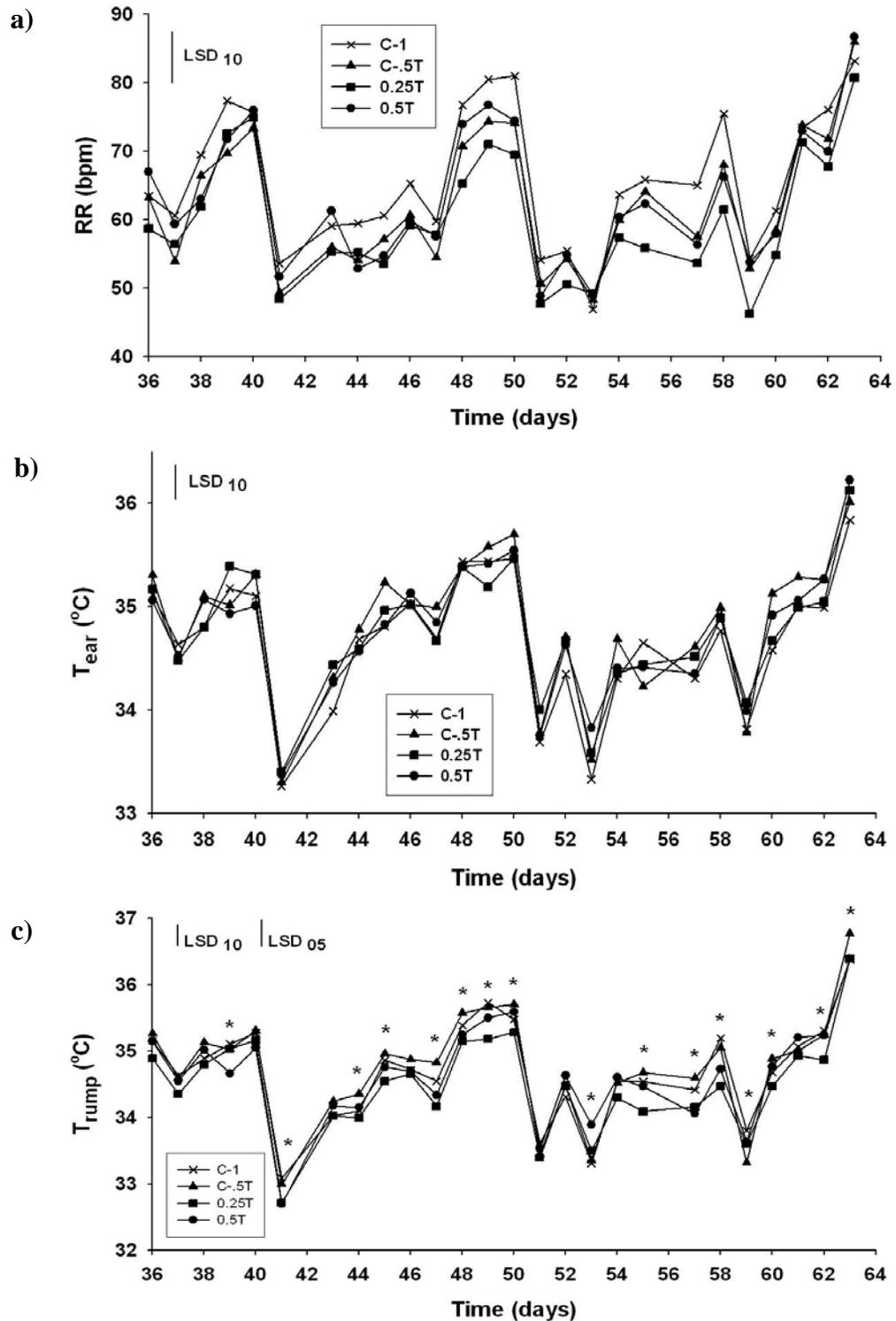
Figure 2.15. Influence of Tasco on daily average of thermal status during Period 3. They include: a) respiration rate (RR); b) ear skin temperature (T_{ear}); c) rump skin temperature (T_{rump}). (*) Days with differences at 5 or 10% between one or more treatments; see text for details.

a)

b)
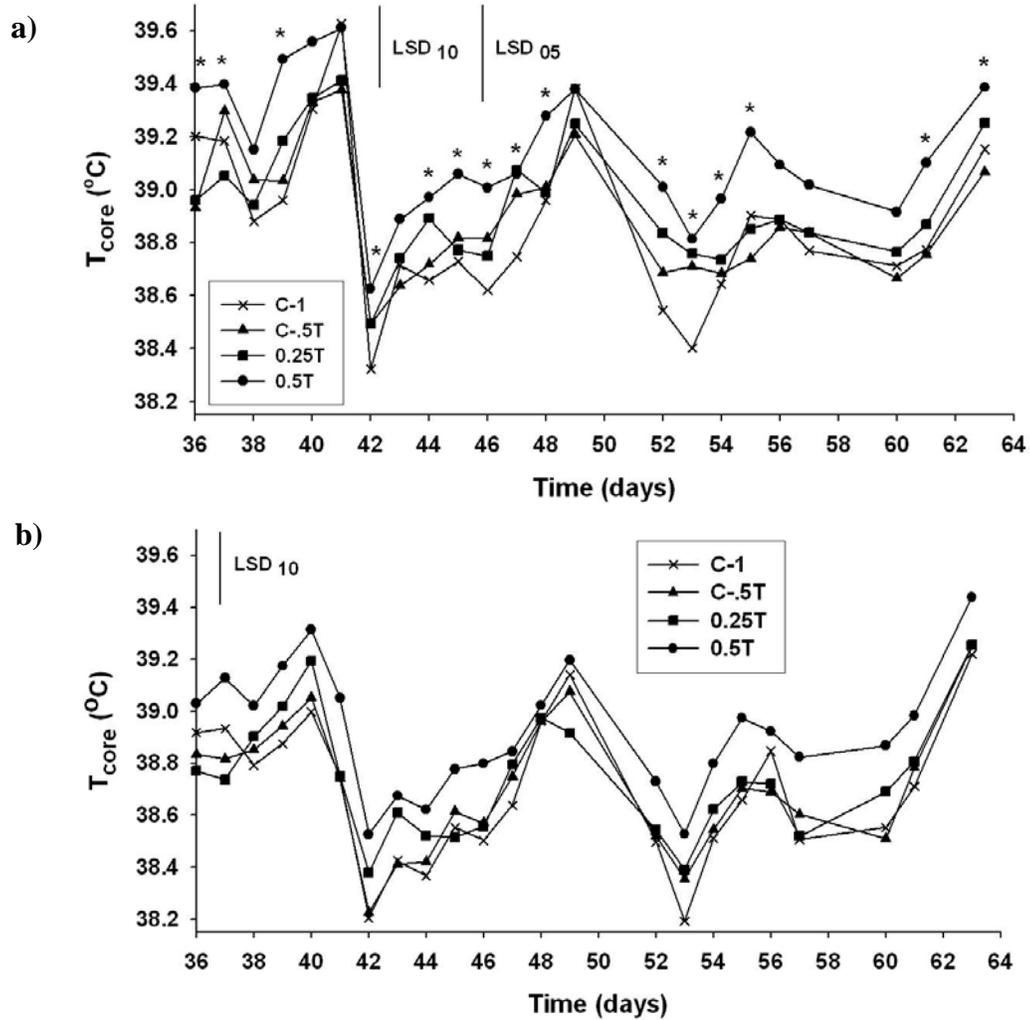
Figure 2.16. Influence of Tasco on daily average core body temperature (T_{core}) during Period 3. They include: a) average T_{core} during the nighttime range; b) average T_{core} during the daytime range. (*) Days with differences at 5 or 10% between one or more treatments; see text for details.

Table 2.7. Influence of Tasco on performance parameters during Period 3: DMI, milk production and composition, milk efficiency, body weight, and BCS

Item	Trt				SE$_{pooled}$	P value
	C-1	C-.5T	0.25T	0.5T		
Cows, n	8	8	8	8		
DMI, kg/d	24.31	22.87	21.81	22.64	0.89	0.26
DMI per BW, kg/kg	0.038	0.036	0.035	0.036	0.0017	0.61
Milk, kg/d	30.11	28.03	28.67	29.77	1.71	0.82
Milk components						
Fat, %	3.38	3.69	3.53	3.60	0.16	0.58
Fat yield, kg/d	0.99	1.01	0.99	1.05	0.06	0.87
Protein, %	2.93	2.87	2.90	2.97	0.09	0.85
Protein yield, kg/d	0.86	0.80	0.82	0.87	0.04	0.66
ECM, kg/d	29.29	28.09	28.75	30.13	1.51	0.81
ECM/DMI, kg/kg	1.22	1.24	1.33	1.34	0.06	0.43
SCS	3.99	2.34	3.87	2.46	0.68	0.20
BW change, kg	-7.5	6.5	1.9	3.5	5.3	0.23
BCS change	0.03	0	-0.09	-0.13	0.07	0.41

[1] ECM = energy-corrected milk = (0.327 x milk yield [kg/d]) + (12.95 x fat yield [kg/d]) + (7.2 x protein yield [kg/d]);
[2] ECM/DMI = milk efficiency;
[3] SCS = SCC linear score = $\log_2$ (SCC/100,000) + 3.

Table 2.8. Influence of Tasco on thermal status during Period 3: respiration rate (RR), ear (T_{ear}) and rump skin temperatures (T_{rump}) in the morning, afternoon, evening, and daily averages, and core body temperature (T_{core}) for daily minimum, maximum, and change, and average for nighttime and daytime ranges

Item	Trt				SE_{pooled}	P value
	C-1	C-.5T	0.25T	0.5T		
RR, bpm						
0700 h	59.36	55.32	53.14	56.16	2.72	
1600 h	71.93	70.04	66.35	69.91	2.72	0.53
1900 h	66.86	61.88	60.04	63.02	2.72	
Daily average	66.05	62.41	59.84	63.03	2.66	0.35
T_{ear}, °C						
0700 h	34.34	34.52	34.50	34.40	0.08	
1600 h	35.09	35.23	35.11	35.18	0.08	0.54
1900 h	34.55	34.73	34.65	34.68	0.08	
Daily average	34.66	34.83	34.75	34.76	0.08	0.47
T_{rump}, °C						
0700 h	34.04	34.13	33.93	33.97	0.09	
1600 h	35.18	35.24	34.88	35.13	0.09	0.25
1900 h	34.87	34.91	34.65	34.82	0.09	
Daily average	34.70	34.76	34.48	34.64	0.08	0.14
T_{core}, °C						
Entire day						
Min	37.87	37.84	37.85	38.11	0.13	0.38
Max	39.67	39.59	39.62	39.84	0.12	0.47
Change[1]	1.80	1.75	1.77	1.73	0.07	0.92
Nighttime[2]	38.88	38.89	38.94	39.14	0.12	0.36
Daytime[3]	38.68	38.70	38.72	38.92	0.11	0.36

[1] Change = max – min;
[2] Range of hours from 0 to 0600 h;
[3] Range of hours from 0700 to 1600 h.
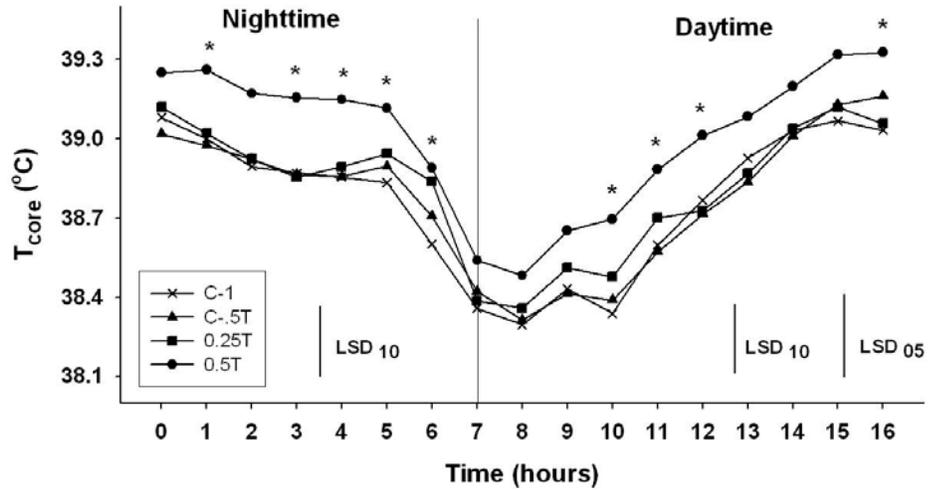
Figure 2.17. Influence of Tasco on the hourly average core body temperature (T_{core}) during nighttime and daytime for Period 3. (*) Hours with differences at 5 or 10% between one or more treatments; see text for details.
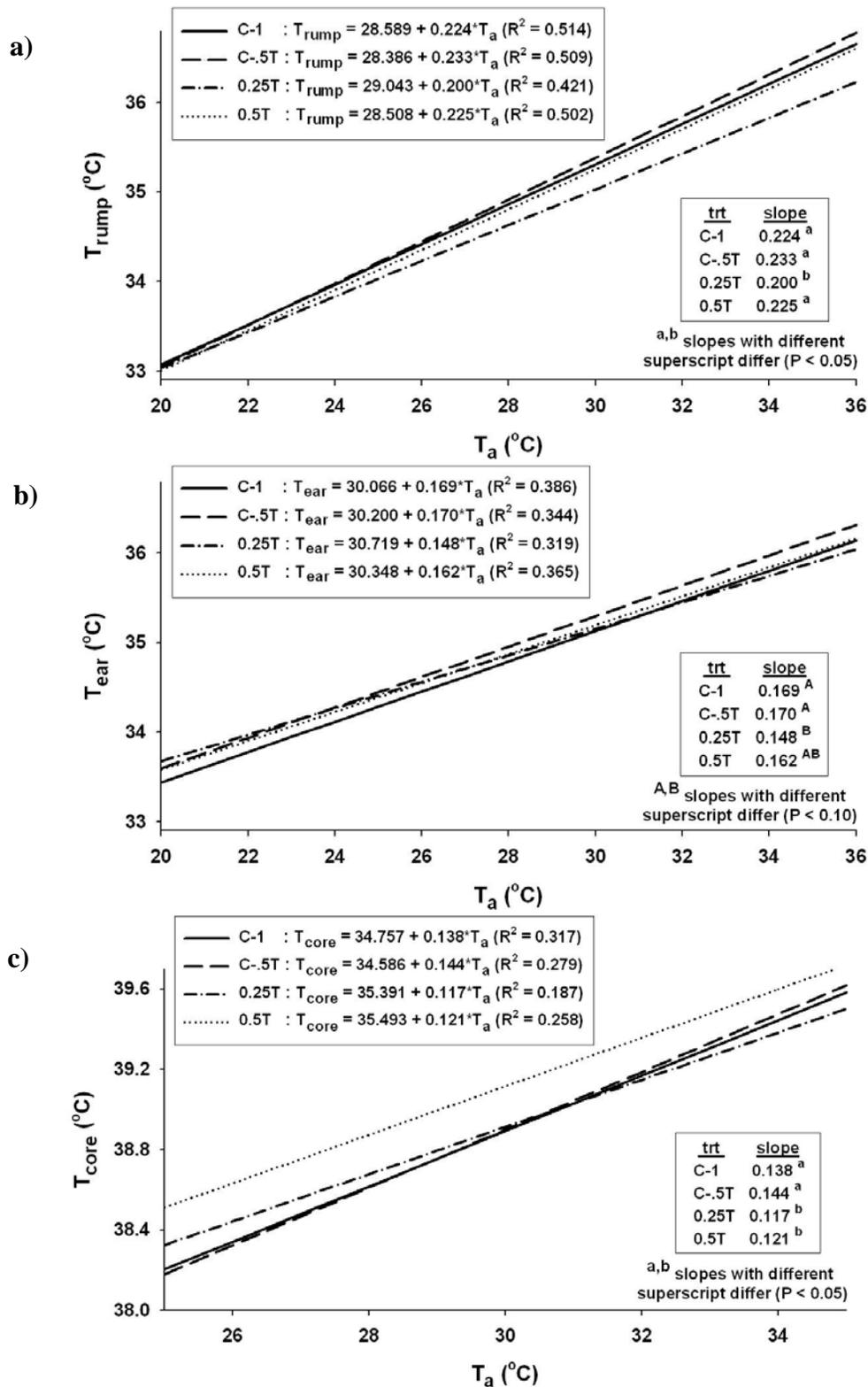
Figure 2.18. Influence of Tasco on the change of thermal status as functions of ambient temperature (T$_a$) during Period 3. They include: a) rump skin temperature (T$_{rump}$) vs. T$_a$; b) ear skin temperature (T$_{ear}$) vs. T$_a$; c) core body temperature (T$_{core}$) during the daytime vs. T$_a$.

92

CHAPTER 3

HEAT STRESS EFFECTS ON THERMAL RESPONSE AND

PERFORMANCE OF DAIRY COWS

ABSTRACT

The present study was performed to identify heat stress effects on thermal status and performance of dairy cows. Holstein cows (n = 15; parity 2.7 ± 1.5; DIM 138 ± 50) were housed in a free-stall barn. The study lasted 63 days during June and July, 2008; in the first 7 days, cows were adapted to the Calan Gate system. Core body temperature (T_{core}) was recorded every 20 minutes with telemetric temperature transmitters (SmartStock, Pawnee, OK) placed in the reticulum. Respiration rate (RR), measured by enumeration of abdominal movements for 30 seconds, and rump (T_{rump}) and ear (T_{ear}) skin temperatures, measured with an infrared heat gun (Raytec, Everett, WA), were collected daily at 0700, 1600, and 1900 h. Ambient temperature (T_a) and relative humidity were recorded every 15 minutes (Hobo, Onset Computer Corp., Bourne, MA). Feed intake and milk production were recorded daily. Heat stress and thermoneutral periods were selected and compared, and a phase of progressively increasing heat stress was evaluated. Heat stress reduced (P < 0.0001) feed intake and milk production, and elevated (P < 0.0001) respiration rate, surface and internal body temperatures of dairy

cows. The increase in respiration rate, skin and core temperatures relative to the increase in ambient temperature was greater ($P < 0.05$) during heat stress than during thermoneutrality. During the progressive heat stress phase, T_{rump} was the thermal parameter that had most of the variation explained by T_a ($R^2 = 0.531$). The variation in T_{core} was most explained by RR ($R^2 = 0.328$), T_a ($R^2 = 0.327$) and T_{rump} ($R^2 = 0.322$). Minimum T_a had more influence on the decline of DMI and milk production during heat stress compared to maximum or mean T_a. However, only a small part of these declines were explained by T_a(min) ($R^2 = 0.084$ for DMI, and $R^2 = 0.049$ for milk production), indicating that many other factors contribute to the negative impact of heat stress on performance. Sensitive and non-sensitive animals within the population were compared. Overall averages for DMI, milk production, RR, T_{ear}, T_{rump} and T_{core} did not differ ($P > 0.10$) between groups. However, animals differed in their response to heat stress, either by utilizing different mechanisms to cope or by not responding to it. If the most sensitive animals were to be identified within the group, preventive and corrective actions could be taken to reduce heat stress effects on the whole herd.

INTRODUCTION

Heat stress is a major concern for the US livestock industry, since it causes considerable losses every year, related to decrease in performance (production, efficiency and reproduction), health and well-being, and increase in mortality of animals. Losses due to heat stress are estimated to be $ 2.4 billion dollars per year for the total livestock industry, with the dairy industry contributing $ 1.5 billion dollars (St-Pierre et al., 2003). Dairy cows are very susceptible to heat stress because of the metabolic heat produced

(Kadzere et al., 2002), added to the difficulty to lose heat to the environment when ambient conditions are unfavorable (i.e. high ambient temperature and relative humidity) (West, 2003). The temperature-humidity index (THI) is commonly utilized to assess ambient conditions causing heat stress in animals, but some researchers suggested that this tool may not accurately represent the heat load for animals (Berman, 2005; Mader et al., 2006). So, an alternative and probably more effective way to assess heat stress and predict its impact on production, is by measuring the animal strain in response to the ambient condition, such as respiration rate and body temperature. Some authors suggested that respiration rate is a better indicator of heat stress, because it is sensitive, easy to monitor and affected before any response in body temperature (Brown-Brandl et al., 2005b; Spiers et al., 2004b). So, the objective of this study was to identify the effects of ambient conditions during heat stress on dairy cow's thermal responses and to evaluate how animal's performance parameters are affected by ambient conditions and animal's thermal status. Moreover, the possibility of predicting loss in productive performance of dairy cows through the animal's own response was analyzed under fluctuating ambient temperature and humidity, during summer heat stress.

MATERIALS AND METHODS

Animals and experimental procedures

This study was performed to determine heat stress effects on dry matter intake (DMI), milk production, and animal's thermal status, as well as to evaluate the possibility of predicting loss in production due to heat stress by analyzing ambient conditions and animals responses. This study was part of another study, which was performed to

evaluate the effects of heat stress on dairy cows (n = 32) supplemented with 0, 0.25, or

0.50% Tasco (*Ascophyllum nodosum*) in the diet, during 63 days in June and July, 2008.

Sixteen cows received a control diet (no Tasco) for the first 35 days of the study and 8 of

these cows received 0.50% Tasco from day 36 to 63. No effect of Tasco was observed for

these 8 cows for any parameter analyzed; so, the 16 animals, except for one, with a mal-

functioning temperature transmitter, were utilized in the current study. Animals fed the

Tasco diets for 56 days were not included in the study.

Cows (milk production = 34.2 ± 5.0 kg/d, ranging from 25.2 to 42.4 kg/d; parity =

2.7 ± 1.5; DIM = 138 ± 50)) were housed in free-stall barn at the University of Missouri

Foremost Dairy Research Center (Columbia, MO). The Animal Care and Use Committee

at the University of Missouri approved the protocol for conducting this study.

Feeding and milking managements

During the first 7 days, cows were acclimated to the Calan Gate system. Animals

were fed a TMR diet, consisting of corn silage/alfalfa haylage and corn. Cows were fed

individually at 0800 and 1400 h, and daily feed intake was recorded for each cow. Cows

were milked twice daily at 0500 and 1700 h, following procedures recommended by the

National Mastitis Council (2002). Milk production of each cow was recorded

electronically at each milking (Metatron 12; Westfalia, Elk Grove Village, IL).

Ultimately, the morning and afternoon production were combined to have a daily milk

production.

Analytical procedures for feed samples

Daily feed samples were collected and combined by week. Weekly samples were

ground through a Wiley Mill (Thomas Scientific, Swedensboro, NJ) using a 2 mm screen,

and analyzed in duplicate for DM (AOAC Official Method 934.01, 1995), NDF and ADF (Van Soest et al., 1991). Total nitrogen was determined by combustion analysis (LECO Instruments, Inc., St. Joseph, MI; AOAC Official Method 990.03, 1995) and the CP was obtained by multiplying the total nitrogen by 6.25. The composition and chemical analysis of the TMR diet are shown in Table 3.1.

Thermal status measurements

Respiration rates and skin surface temperatures. Thermal status measurements (skin temperature and respiration rate) were taken daily at 0700, 1600, and 1900 h. Skin temperature was measured in the rump (T_{rump}) and ear (T_{ear}), using a calibrated infrared heat gun (Raytec, Everett, WA). Respiration rates were measured by enumeration of abdominal movements for 30 seconds.

Core body temperature. Core body temperature (T_{core}) was recorded using telemetric temperature transmitters (SmartStock, Pawnee, OK) inserted orally using a bolus gun to lodge in the reticulum of each cow. The transmitters were approved by FDA and do not need to be removed. The telemetric system was composed by a bolus, antenna, barn receiver unit, base receiver unit, and a computer, installed with a software program for logging the data, and located inside a building, about 30 meters away from the free-stall barn. The antenna for transmitting data to the base receiver unit was located in the center of the barn where cows were housed, above the free-stalls, about 3 meters of the floor. The telemetric bolus (3 cm in diameter, 8.5 cm in height, and 120 g in weight) was programmed to record temperature every 20 minutes, with each transmission containing 12 readings. Because the temperature of the rumen is reduced with water intake (Boehmer et al., 2009), a maximum value of T_{core} per hour was obtained from the 3

97

recordings in order to eliminate the decrease in reticulum temperature due to water consumption. Thus, each day was composed of 24 T_{core} values representing each hour. A power outage caused loss of T_{core} data for the following days: 1, 3, 11, 12, 23, 50, 51, 58, 59 and 62, resulting in the removal. Moreover, a test for outliers was performed for T_{core} vs. T_a utilizing quadratic regression; for each animal, T_{core} observations that were greater than 2.5 standard deviations from the predicted value were removed from the data set. This new data set, with the outliers removed, was utilized for all the analysis of T_{core}.

For the analysis of T_{core}, entire days and ranges of hours within days were analyzed. For the analysis of the whole day, when T_{core} was evaluated as a function of T_a, the milking times (from 0500 to 0700 h and from 1700 to 1900 h; Figure 3.1) were removed from the data set, because ambient conditions at the milking parlor were not recorded. In order to represent the longest and most sustained rise in T_{core} during the day, a range of hours from 1000 to 1500 h was analyzed (Figure 3.1). There were two extremes for low T_{core} during early morning, the first happening around 0730 h, and the second around 1000 h. It was speculated that the small increase in T_{core} between 0730 and 0900 h (Figure 3.1) was attributed to the feeding time, when a fresh load of feed was offered to the animals, stimulating intake and increasing heat production by rumen fermentation (Kadzere et al., 2002). Thus, 1000 h was preferred over 0700 h to represent the start of the rise in T_{core} of the day, in order to remove the oscillation in T_{core} related to the feeding, from 0730 to 1000 h. Also, there was a drop in T_{core} after 1500 h, so, this hour was chosen to be the ending of the range. Moreover, for those analysis in which T_{core} was compared or related to RR, T_{ear} or T_{rump}, the data set for T_{core} was summarized

in only 3 hours each day (0700, 1600 and 1900 h), corresponding to the hours when RR, T_{ear} and T_{rump} were collected.

Ambient conditions measurements

Ambient temperature (T_a) and relative humidity (RH) were recorded automatically every 15 minutes using data loggers (Hobo, Onset Computer Corp., Bourne, MA) placed in three different locations in the free-stall barn. Two of the loggers were placed in the center of the barn, above the free-stalls, about 3 meters of the floor; the other logger was placed in the mid-point above the Calan Gate feeding system, 3 meters of the floor. Hourly average T_a of the three data loggers was calculated and utilized in the analyses. For RH, only one data logger collected useful data, so, the records for the other two were eliminated. The THI was calculated hourly by the formula: THI = (T_a x 0.81) + RH(T_a – 14.4) + 46.4, where T_a is the dry-bulb temperature (ºC), and RH is the relative humidity (decimals) (Thom, 1959).

Segments of the study for data analysis

From the 63 days of data collection, smaller periods were selected to enable more detailed analyses. When a large number of days are averaged together, most differences disappear because of the large variation within the data set. This is especially true for studies that do not control ambient conditions, and so, a lot of oscillation is present. By utilizing a smaller number of days for this type of data set, undesirable variation is avoided and more realistic results can be observed. The segments selected for a more detailed evaluation are defined as follows.

Six Periods. Six periods were selected within the 63 days, each consisting of two consecutive days (Table 3.2). The first three periods represented thermoneutral

conditions, while the last three represented heat stress conditions. Periods were selected

by visually analyzing the ambient conditions to detect two consecutive days with similar

T_a pattern (Figure 3.2). At least one day in between periods was required in order to make

the periods more independent of each other. Moreover, the day preceding each period

should have the same condition (thermoneutral or heat stress), since carry-over effects

may be expected; this way, each period would be truly represented by, and only by,

thermoneutral or heat stress conditions. The two days in each period were averaged for

each cow in order to obtain one-day values and remove some of the variance. If the

parameter had hourly measurements (i.e. T_{core}, RR, T_{ear}, T_{rump}), the two days were

averaged hourly for each cow. Overall average for performance and thermal parameters

of cows were compared among periods. Moreover, the relationship between T_{core} and T_a

was analyzed to determine whether or not different ambient conditions caused different

changes of T_{core} relative to the increase in T_a. Since each period consisted of one-day

values, it was not possible to analyze the influence of T_a on RR, skin temperature, DMI

and milk production, because of the limited number of data points. The data sets for RR

and skin temperatures consisted of only 3 values (corresponding to measurements at

0700, 1600, and 1900 h), and the data sets for DMI and milk production consisted of only

1 value (corresponding to the daily average).

  Non-HS vs. HS conditions. From the six periods described above, the 3

thermoneutral periods (1, 2, and 3) were combined into the Non-HS group and compared

against the 3 heat stress periods (4, 5, and 6), which were combined into the HS group

(Table 3.2). With that, it was possible to better evaluate differences between animal's

response under thermoneutral and heat stress conditions. Overall averages for

performance and thermal parameters were compared, as well as the relationship between thermal status (RR, T_{ear}, T_{rump}, and T_{core}) and T_a. For this analysis it was possible to analyze all thermal parameters vs. T_a, since the data set was composed of 3 times as many points as the data set for the periods, enabling for more reliable results. However, performance parameters relative to T_a were not possible to analyze, because of the limited number of data points.

Progressive heat stress phase. A phase of progressively increasing T_a was selected from the 63 days of study. This heat stress phase was composed of 9 days (from d 32 to 40; Figure 3.2). In the next day following this phase (d 41), T_a decreased significantly, probably reducing the heat strain of cows, and for this reason, it was opted to end the phase on d 40. However, some days after d 40 were included in the analysis when delays in response were applied, in order to evaluate the carry-over effects of this phase of heat stress on DMI and milk production, which are known to be affected by heat stress with a delay of 0 to 4 days (Maust et al., 1972; Spiers et al., 2004b; West et al., 2003). For beef steers housed in a climatic chamber, exposure to heat stress caused a decrease in DMI after 3 to 4 days (Williams et al., 2009). One cow was diagnosed with mastitis during this phase and was removed from the data set; thus, the progressive heat stress phase consisted of 14 animals. Relationship between animal's thermal status and performance parameters vs. T_a, as well as the relationship between the animal's parameters, were tested in an attempt to determine any relationship during this progressive heat stress phase.

Sensitivity to heat stress. After the analysis, it was clear that a lot of variation occurs within a population of animals (Berman et al., 1985; Mader et al., 2009), and

makes the results challenging to interpret, influencing the accuracy. For many cases, regressions between animal parameters yielded a low coefficient of determination (R^2) when it was expected to be high. Many factors may cause animals to be more or less sensitive to heat, such as level of milk production, body size, breed, coat color, temperament, parity, genetic diversity, health and nutritional status (Hahn, 1999; Spiers et al., 2004b). Some animals respond more quickly to changes in the ambient conditions, while others respond more slowly; for some animals, heat stress may have an enormous effect, while for others it has no effect at all. If the most sensitive animals are identified, producers can handle them differently, with a lower density diet and management programs, reducing the heat stress effects on the whole herd. Thus, in order to determine if the low R^2 obtained for analyses were attributed to the large variation in response within the population, animals were separated into two groups, sensitive (S) and non-sensitive (NS). Sensitivity of animals was assessed during the progressive heat stress phase (d 32 to 40). By utilizing such a phase, animal response to a wide range of ambient temperatures could be identified. Thermal and performance responses of animals were evaluated in order to define the groups. The R^2 values for individual animals, for the linear regression of T_{core} vs. T_a and DMI vs. minimum T_{core} ($T_{core}(min)$), were utilized to rank animals by responsiveness. For the relationship between T_{core} and T_a, data points from 1000 to 1500 h were utilized in order to express the major rise in T_{core} during the day, starting at the minimum T_{core}, which happened generally at 1000 h. Also, milking times were avoided, because T_a was not collected in the parlor. Moreover, it was found that, during the progressive heat stress phase, the relationship between T_{core} and T_a was linear with a 2 hour delay in response (Table 3.7), and this delay was utilized for the

analysis of sensitivity. For DMI, the best relationship found was a linear regression with no delay, utilizing the minimum T_{core} of the day (Table 3.10), which was utilized in the sensitivity analysis. Cows were ranked by the R^2 value for each relationship, and the most responsive cows for both parameters were assigned to the S group (n = 5), while the least responsive for both parameters were assigned to the NS group (n = 5). The 4 extra cows which showed an intermediate response were removed from the analysis, in order to have a better separation between the two groups. Other authors have utilized a different approach to separate animals relative to their sensitivity (Mader et al., 2009). Maximum tympanic temperature during the hottest day of a 6-day period was utilized to separate beef steers into high (> 41.7°C), moderate (41.3 to 41.7°C), and low (< 41.3°C) tympanic temperature profile.

Data analysis

For all parameters analyzed, only healthy animals were utilized. Cows with mastitis for a certain period during the study were removed from the data set for that specific period of sickness and placed back after they recovered. One cow was removed from periods 1 and 2; another cow was removed from period 3, 4, 5, and from the progressive heat stress phase; a third animal was removed from period 6.

The data were analyzed as a complete block design with repeated measurements over time for respiration rate, ear and rump surface temperature, T_{core}, DMI and milk production (Littell et al., 1998). Each cow was considered as a complete block and all cows were evaluated in all time periods. Comparison between parameter averages, for the analysis of the six periods, Non-HS vs. HS, and sensitivity, was assessed by LSMEANS function of MIXED procedure of SAS (SAS Institute Inc., Cary, NC). For RR, skin and

core temperatures, the interaction trt x h was also analyzed. In order to determine

relationship among animal's parameters, and between parameters and ambient

conditions, PROC REG of SAS was utilized to fit linear and quadratic regressions and to

find equations and R^2 for each relationship. The THI (or RH) were not included in the

analysis because T_a and THI were highly correlated ($R^2 = 0.94$, as a fit of all 63 days),

indicating that T_a alone could describe effectively the ambient conditions. The linear

regression was preferred if the quadratic was not significant ($P > 0.05$) or if the shapes of

the linear and quadratic curves were similar. For most of the cases, when the linear and

quadratic curves were compared, the 95% confidence interval (CI) of both curves

overlapped, suggesting that the linear function could be utilized to express the

relationship without losing quality in the analysis, and that the quadratic function would

not improve significantly the relationship between parameters. Differences between

slopes were detected by the GLM procedure of SAS. Furthermore, the delay in the

response of one parameter to another was tested in order to determine the best lag time,

which was verified by the highest R^2 for the linear or quadratic regression. This same

approach has been used by other researchers (Brown-Brandl et al., 2005a; Brown-Brandl

et al., 2005b) to determine the most appropriate lag for RR and T_{core} relative to dry-bulb

temperature and solar radiation. Animal was the experimental unit, and $P < 0.05$ was

utilized for establishing significant differences.

RESULTS AND DISCUSSION

Six Periods

Ambient conditions during the six periods. Figure 3.2 shows the T_a for the six periods selected; Figure 3.3 shows the average hourly T_a for the two days within each period. On average, thermoneutral periods (1, 2 and 3) had a 4°C fold lower temperature than the heat stress periods (4, 5 and 6). For all periods, minimum T_a occurred around 0600 h and maximum T_a occurred around 1500 h. The mean T_a for each period, as well as the average minimum and maximum, are show in Table 3.3. All periods differed ($P < 0.0001$) from each other for mean, minimum and maximum T_a. Period 6 had the highest mean, minimum and maximum T_a, while period 3 had the lowest mean and maximum T_a and period 1, the lowest minimum T_a (Table 3.3).

Performance parameters during the six periods. Average for production parameters for cows during each period are shown in Table 3.3. Differences ($P < 0.0001$) were found between periods for DMI and milk production. During period 2, cows had the highest ($P < 0.001$) DMI, followed by periods 1, 3 and 6 ($P < 0.01$). The lowest ($P < 0.05$) DMI was observed in periods 4 and 5. The DMI was expected to be higher for the thermoneutral periods, and lower for the heat stress periods, since under hot conditions, cows reduce their feed intake in order to avoid an overload of heat production due to the heat of digestion and fermentation, and thus, maintain a lower T_{core} (Hahn, 1999). However, contrary to our expectations, period 6, which had the highest T_a, had DMI as high as periods 1 and 3, which had the lowest T_a. Possibly, by period 6, cows were adapting to the elevated T_a, which started around period 4, causing DMI to be increased. Other studies have shown cattle acclimating to heat stress (Brown-Brandl et al., 2005a;

Hahn et al., 1990). When *Bos taurus* beef cattle were housed in environmentally controlled chambers, acclimation to heat stress occurred after 3 to 4 days of exposure to hot conditions, and at this point, T_{core} started to decrease and feed intake increased (Hahn et al., 1990). After 8 days of heat exposure, physiological adjustments appeared to be completed (Hahn et al., 1990). Other authors also found beef steers exposed to simulated cyclic T_a acclimated to heat stress after 3 to 4 days (Brown-Brandl et al., 2005a).

Milk production was not different (P > 0.10) between thermoneutral periods 1, 2, and 3, or between heat stress periods 4, 5, and 6. In period 2, cows had a higher (P < 0.05) milk production compared to any of the heat stress periods, while during periods 1 and 3, milk production was not different (P > 0.10) than period 4. In periods 5 and 6, milk production was lower (P < 0.01) than all thermoneutral periods. A lower milk production is expected during heat stress periods, due to reduced feed intake, thus decreasing energy intake and the energy available for milk production (Kadzere et al., 2002), even though some authors have speculated that only 35% of the decline in milk production during heat stress is attributable to lower feed intake (Rhoads et al., 2010; Rhoads et al., 2009). Since the periods were spread out within 34 days and by the first period cows had long passed the peak lactation, it is likely that by the last period, a natural decline in milk production had occurred. The average days in milk (DIM) for the first period was 152 days, while for the last period, it was 185 days; thus, milk production could be expected to decrease by 2.6 kg from the first to the last period (DHI Info sheet). The decrease from period 1 to period 6 was only about 2 kg, which agrees with the actual decline in milk production. Therefore, it is difficult to conclude if the reduction in milk production observed from the first to the last period was attributed to DIM or heat stress.

However, comparing periods 3 and 5, which were only 8 days apart, cows had an average of 2 kg lower ($P < 0.0001$) milk production in period 5 than in period 3. This indicates that the reduction in milk production in period 5 could be related to heat stress. Also, the observed increase in RR and T_{core} for cows in periods 5 and 6 indicates cows were heat stressed, possibly causing milk production to be reduced.

   Thermal status during the six periods. Thermal status of cows during each period is shown in Table 3.3. Differences ($P < 0.0001$) existed between periods for overall average RR, T_{ear}, T_{rump} and T_{core}. Average RR was higher ($P < 0.0001$) for periods 5 and 6 compared to all other periods. In period 4, cows had lower ($P < 0.0001$) RR than periods 5 and 6, but higher ($P < 0.001$) than all the thermoneutral periods. The lowest ($P < 0.001$) value of RR was observed during period 3. A higher RR is expected when T_a is high, to increase dissipation of body heat and reduce the elevation of T_{core}. The RR is the first response to hot conditions, followed by evaporative skin loss. If the heat loss through the respiratory system and skin cannot dissipate the heat load accumulated in the organism, T_{core} will increase (Berman, 2005). Average skin temperatures (ear and rump) were higher during period 6 ($P < 0.05$), followed by period 5 ($P < 0.05$), and next 4 ($P < 0.05$). In period 3, cows had the lowest ($P < 0.0001$) T_{ear} and T_{rump} of all periods. Rump temperature was not different ($P > 0.10$) between periods 1 and 2, but T_{ear} was higher ($P < 0.05$) for period 1. The increase in skin temperature, especially in the periphery, indicates that blood flow from the core to the skin was increased, as an attempt to dissipate internal heat through the skin (Armstrong, 1994). Moreover, skin temperature may be directly affected by ambient conditions, thus, if the ambient temperature increases, skin temperature may also rise (Collier and Zimbelman, 2007).

Core body temperature, as analyzed for the whole day, was higher ($P < 0.01$)

during periods 5 and 6, followed by period 4. As expected, during the 3 thermoneutral

periods, cows had the lowest T_{core} ($P < 0.0001$). When this parameter was analyzed only

from 1000 to 1500 h, cows in periods 1, 2, and 3 exhibited lower ($P < 0.0001$) T_{core}, while

those in periods 4, 5 and 6 were higher. No differences ($P > 0.10$) were found between

thermoneutral period 1, 2, and 3, or between heat stress periods 4, 5, and 6. A higher

internal body temperature is expected under heat stress conditions, if the heat load in the

body is greater than the heat loss from the body, such as through respiratory or cutaneous

evaporative heat loss (Kadzere et al., 2002), suggesting cows were heat-stressed.

Hourly average of T_{core} for each period is shown in Figure 3.4. During the early

hours (from 0 to 0500 h), cows in the thermoneutral periods had a $0.4^{o}C$ fold lower T_{core}

than cows in the heat stress periods. Later in the day (after 1500 h), this difference was

increased to almost $1.0^{o}C$. There was a drop in T_{core} during the afternoon hours in period

4, deviating from the other heat stress periods, probably because of rain in one of the two

days that composed this period. The result was a drop in T_a (Figure 3.3) and a decrease in

T_{core} to the level seen in thermoneutral periods after 1800 h. The minimum T_{core} occurred

at 0700 h for periods 1, 2, and 5, 0800 h for period 6, and 1000 h for periods 3 and 4. In

general, every period had two drops in T_{core} during the early morning, the first one around

0730 h and the second around 1000 h. After a rapid rise from 1000 to about 1500 h, T_{core}

stabilized at the high temperature, and was maintained almost constant until the end of

the day and beginning of the next day. Maximum values for T_{core} occurred between 2200

and 0100 h for all periods, except for period 2, when T_{core} peaked at 1500 h. Kendall et al.

(2006) reported a minimum vaginal temperature of cows in New Zealand occurring

around 0710 to 0720 h, while peak temperature was observed around 1720 and 1730 h. Cows in that study were housed outdoors in a grazing system, what can explain the earlier peak in body temperature compared to the current study.

In the present study, even though hourly T_{core} was generally lower ($P < 0.05$) for the 3 thermoneutral periods compared to the 3 heat stress periods (Figure 3.4), the rate of change in T_{core} as a function of time was similar for almost the whole day ($P > 0.10$), except between 1000 to 1500 h, which corresponded to the major rise in T_{core} during the day. During this time, the rate of increase in T_{core} was higher ($P < 0.05$) for the heat stress periods 5 and 6, compared to the thermoneutral periods 1 and 2. In period 3, however, the rate of increase in T_{core} from 1000 to 1300 h was not different ($P > 0.10$) from those of the heat stress periods 4, 5, and 6, and after that time, the behavior was similar ($P > 0.10$) to the other thermoneutral periods. At 1000 h, the absolute value for T_{core} in period 3 was lower ($P < 0.05$) than all other periods, but with the fast increase from 1000 to 1300 h, it reached same levels of T_{core} seen in the other thermoneutral periods by 1300 h.

Regression of thermal status with ambient temperature for the six periods.
Linear and quadratic regressions of T_{core} as a function of T_a for each period, with different delays in response, are shown in Table 3.4. For the whole day, a delay in response of T_{core} to T_a of about 4 hours existed, as indicated by the relationship having the highest R^2. When *Bos taurus* beef steers were housed in environmentally controlled chambers, tympanic temperature lagged air temperature by about 2 to 5 hours (Hahn et al., 1990). In the present study, the response of T_{core} to T_a was quadratic, as indicated by a higher R^2 compared to the linear function, for all periods. However, R^2 values were relatively low for all relationships, indicating that only a small portion of the variation in T_{core} was

109

explained by T_a, especially during the thermoneutral periods. This ranged from 4.4% in period 2, to 34.7% in period 5. When analyzed from 1000 to 1500 h, T_{core} responded linearly to the change in T_a, with no delay in response, as indicated by the highest R^2. It is interesting that, when only 1000 to 1500 h was analyzed, T_{core} responded immediately to T_a. However, when data for the whole day was evaluated, it took an average of 4 hours for T_{core} to respond to T_a. Thus, when lower values of T_a are incorporated in the analysis (i.e. whole day), the response of T_{core} to the change in T_a is delayed, probably because below a certain threshold (i.e. 25ºC) T_{core} does not depend on T_a. For period 6, almost half (46.4%) of the variation in T_{core} from 1000 to 1500 h was explained by T_a; on the other hand, for period 1, T_a explained only 5.7%. This indicates that T_a had a greater influence on the variation of T_{core} under heat stress (i.e. period 6) compared to thermoneutral conditions (i.e. period 1), when T_{core} is not dependent on T_a.

Figure 3.5a presents the quadratic regression for each period, using a 4 hours delay, for T_{core} (whole day) as a function of T_a. For periods 1, 2, and 3, T_{core} did not change much with the change in T_a, what was expected, since at thermoneutrality T_{core} is almost constant and does not depend on T_a (Berman et al., 1985). On the other hand, for periods 4, 5, and 6, T_{core} increased up to a certain level, and then stabilized with further increase in T_a. The linear regression of T_{core} (1000 to 1500 h) vs. T_a, with no delay for each period is shown in Figure 3.5b. The slopes for periods 3, 4, 5, and 6 did not differ (P > 0.05), thus, T_{core} increased at the same level for these periods with the increase in T_a. Periods 1 and 2 had a lower slope than all other periods, meaning T_{core} increased less with increasing T_a during these two periods compared to others. The R^2 (Table 3.4) and the slopes (Figure 3.5b) for periods 1 and 2 were very small, although significant. This was

expected, since internal body temperature does not depend on T_a when it is below 25ºC (Berman et al., 1985), and so, a poor relationship was expected for the thermoneutral conditions. However, the opposite happened during the thermoneutral period 3, when a low relationship was also expected, but instead a large R^2 and slope as great as the slope for heat stress periods 4, 5, and 6 were obtained. As described earlier in Figure 3.4, the minimum T_{core} of cows at 1000 h in period 3 was significantly lower than all other periods, even the other thermoneutral periods, and probably the rate of increase of T_{core} was much faster in order to catch up with the other periods, resulting in a greater slope.

Non-HS vs. HS conditions

Ambient conditions during Non-HS and HS conditions. As shown in Table 3.5, ambient conditions, performance and thermal parameters were compared between Non-HS (represented by periods 1, 2, and 3; Table 3.2) and HS (represented by periods 4, 5, and 6; Table 3.2). The hourly average T_a for each condition is shown in Figure 3.6. The T_a pattern for the two conditions was very similar throughout the day, with minimum T_a occurring around 0600 to 0700 h, and maximum occurring at 1500 h. There was a 6^oC difference between the conditions for virtually every hour, with HS having higher T_a. Moreover, the mean, minimum and maximum T_a were different ($P < 0.0001$), and, as expected, higher for HS (Table 3.5).

Performance parameters during Non-HS and HS conditions. Differences ($P < 0.0001$) in DMI and milk production were found between Non-HS and HS. Both were lower during HS compared to Non-HS, as expected, since a decline in intake generally occurs when cows are exposed to heat stress, with a consequent drop in milk production (Hahn, 1999; Kadzere et al., 2002).

Thermal status during Non-HS and HS conditions. The RR, T_{ear}, T_{rump} and T_{core} (whole day and 1000 to 1500 h) were higher (P < 0.0001) for HS vs. Non-HS. Respiration rate is increased under heat exposure as an attempt to dissipate heat; also, heat flow to the skin is increased, elevating skin temperature; when dissipation of heat by respiratory and cutaneous avenues are not enough, internal body temperature increases, indicating that the cows are heat-stressed (Kadzere et al., 2002).

As shown in Figure 3.7, a 0.4^{o}C fold difference in T_{core} existed between the Non-HS and HS conditions during the early morning. However, this difference increased to about 0.7^{o}C during the hottest time of the day. The rate of change in T_{core} as a function of time was not different (P > 0.10) between Non-HS and HS conditions for almost the whole day, except from 1000 to 1500 h, when T_{core} for HS increased faster (P < 0.0001) compared to Non-HS. Supporting this finding, Kendall et al. (2006) reported greater differences in vaginal temperature rhythm from 1000 to 1500 h for dairy cows with or without access to shade.

Respiration rate, T_{ear}, T_{rump} and T_{core} were also compared at each time within day (0700, 1600, and 1900 h) when measurements were collected (Figure 3.8). Under HS conditions, an increase (P < 0.05) in RR, T_{ear}, T_{rump} and T_{core} from 0700 to 1600 h occurred and a decrease (P < 0.05) occurred from 1600 to 1900 h. For Non-HS, RR did not differ (P > 0.05) among times; however, T_{ear}, T_{rump} and T_{core} increased (P < 0.05) from 0700 to 1600 h, with no differences (P > 0.05) between 1600 and 1900 h. At thermoneutrality, RR, skin and T_{core} changed less throughout the day compared to heat stress conditions, what was expected, since below a threshold of T_a, thermal status of animals are almost constant (Berman et al., 1985). When comparing the same time within

112

day (0700, 1600 or 1900 h), RR, T_{ear}, T_{rump} and T_{core} were higher (P < 0.05) for HS than Non-HS at all times.

    Regression of thermal status with ambient temperature during Non-HS and HS conditions. The relationship of T_{core} (whole day and from 1000 to 1500 h), RR, T_{ear} and T_{rump} to T_a are shown in Table 3.6. When T_{core} was analyzed during the whole day, there was a delay in the response to T_a of about 4 hours. However, when the variable was evaluated only from 1000 to 1500 h, no delay in response was observed. This difference in delay times was probably attributed to the lack of dependence of T_{core} relative to T_a, when below a threshold, for the analysis of the whole day. In contrast, Kendall et al. (2006) found from 1000 to 1500 h, vaginal temperature of dairy cows lagged behind THI by 1.7 hours. Although cows in that study were housed outdoors, suggesting a higher level of heat stress, the study was done in New Zealand, where moderate air temperatures were experienced by the cows. Thus, the greater delay in that study compared to the current study may be attributed to the lower level of heat stress. In the current study, when the whole day was analyzed, despite a small R^2, it appeared the delay for Non-HS was greater than the delay for HS (5 hours vs. 3 to 4 hours). Supporting these findings, Brown-Brandl et al. (2003) reported a decreased lag during heat stress periods for the response of rectal temperature to changes in air temperature, even though the difference in that case was smaller (4.75 hours in thermoneutral vs. 4 to 4.25 hours in heat stress) compared to the current study. The response of T_{core} to T_a was quadratic for the whole day, because it included lower T_a values, and linear for the 1000 to 1500 h analysis, which represented only the rise in T_{core} during the hottest part of the day.

Only a small portion of the variation in T_{core} was explained by T_a, especially for the Non-HS. For the whole day, T_a explained 3.5 and 17.6% for Non-HS and HS, respectively, and for 1000 to 1500 h, T_a explained 10.2 and 29.9% for Non-HS and HS, respectively. As observed, T_a explained almost twice the variation when T_{core} was analyzed from 1000 to 1500 h, compared to the whole day, what may be attributed to the low T_a included in the whole day analysis. Thus, from 1000 to 1500 h, the contribution of T_a for the variation in T_{core} was greater, and for the other hours, factors other than T_a have a higher effect. For beef steers exposed to heat stress in cyclic and heat waves simulation, T_a explained 29% of the total variation in T_{core}, measured in the abdominal cavity by transmitters, using a linear regression applied to the average daily values of each variable (Brown-Brandl et al., 2005a). Although in the current study the regression was performed with hourly values instead of daily averages, the contribution of T_a to T_{core} was similar to that found by Brown-Brandl et al. (2005a) when 1000 to 1500 h was analyzed. However, for all cases in the present study, the R^2 values were low, suggesting many other factors, besides T_a, had an effect on T_{core}.

Figure 3.9 shows T_{core} as a function of T_a for Non-HS and HS individually, as well as the quadratic regression combining the two conditions. A 4 hour delay was utilized for the whole day plot (Figure 3.9a), and no delay was incorporated for 1000 to 1500 h (Figure 3.9b). Slopes for T_{core} vs. T_a were higher ($P < 0.05$) for HS compared to the Non-HS for both whole day and from 1000 to 1500 h; so, cows had a greater increase in internal body temperature under elevated T_a compared to cooler conditions. In agreement, Kendall et al. (2006) found that the slope for the relationship between vaginal temperature and THI was 0.3ºC for cows with access to shade, and 1.2ºC for cows with

no access to shade, indicating the increase in vaginal temperature was greater for cows exposed to heat stress. In the current study, for T_{core} (from 1000 to 1500 h) vs. T_a (Figure 3.9b), T_{core} undershot at cooler temperatures during HS, dropping to values lower than T_{core} during Non-HS, for the same value of T_a. One reason for that is the higher necessity for cooling at night which happens during heat stress periods. When the change in T_{core} was higher, the decline was greater, and a lower value of T_{core} was reached. During Non-HS, change in T_{core} was small, thus cows did not need to have a large drop in T_{core} at night to dissipate heat. Moreover, when both conditions were combined for T_{core}, from 1000 to 1500 h, in a quadratic function, T_{core} started to increase after T_a rose above 24°C, agreeing with previous findings (Berman et al., 1985).

From the relationships between RR, T_{ear} and T_{rump} with T_a (Table 3.6), it was noted R^2 for all the parameters was higher for the HS compared to Non-HS condition, which was expected, since at thermoneutrality, the animal's thermal responses are not dependent on ambient thermal conditions, and so, a poor relationship is expected (Berman et al., 1985). Of the variation in RR, T_a explained 7.8 and 37.3% for Non-HS and HS condition, respectively. In contrast, when beef steers were exposed to heat stress in cyclic and heat wave simulation, T_a explained 79% of the variation in RR (Brown-Brandl et al., 2005a). The lower R^2 obtained in the current study may be attributed to the fluctuating ambient conditions that are found in the natural environment, but controlled for, in climatic chambers. Also, Brown-Brandl et al. (2005a) utilized daily averages of the variables in the regression, while in the current study, specific hourly values (at 0700, 1600 and 1900 h) were utilized. In the present study, T_a explained 13.0 and 62.5% of the variation in T_{ear}, and 26.5 and 74.6% of the variation in T_{rump}, for Non-HS and HS

conditions, respectively. As noted, T_a had a higher contribution to the variation of skin

temperature than to the variation in RR. Moreover, T_a had a greater influence on the

variation of T_{rump} compared to T_{ear}. During HS condition, only 25% of the variation in

T_{rump} was explained by factors other than T_a, demonstrating the important relationship

between T_a and skin temperature. Furthermore, no delay in response was found for any of

the variables when the linear regression was tested; however, for quadratic functions, the

delay in response changed with variables and conditions, varying from 0 to 1 hour. This

indicates that the most appropriate lag time for these variables in the current study was

between 0 and 1 hour. Brown-Brandl et al. (2005a) examined beef steers exposed to

simulated cyclic temperature and heat waves, and found an average delay of 1.80 ± 0.12

hours for the response of RR to the change in T_a, and this delay ranged from 0 to 4.5

hours. Moreover, the authors found no differences between thermoneutral and heat stress

for the delay of RR to T_a.

The quadratic regression for RR, T_{ear} and T_{rump} with T_a improved the R^2, even

though this improvement was very small for some variables. However, the shapes of the

linear and quadratic curves were similar. Thus, for simplicity and ease of interpretation

the linear function was preferred over the quadratic. For the graphs of RR, T_{ear} and T_{rump}

as a function of T_a (Figure 3.10) the linear regression for Non-HS and HS was plotted, as

well as the quadratic regression combining the two conditions. No delay in response was

utilized in the plots. When Non-HS and HS conditions were combined together, the

quadratic function was well defined. By visually observation of the plots, it appears that

RR, T_{ear} and T_{rump} were independent on T_a below 22ºC, and as T_a rose above 22ºC, RR,

T_{ear} and T_{rump} started rising. Supporting this finding, Hahn (1999) found a threshold of T_a

for the initial rise in RR to be around 21.3ºC. Slopes for RR (Figure 3.10a), T_{ear} (Figure 3.10b), and T_{rump} (Figure 3.10c) with T_a were greater (P < 0.001) during HS than Non-HS, meaning that the RR and body temperature of cows increased more under elevated T_a compared to thermoneutral conditions. For RR during HS (Figure 3.10a), the use of a linear function underestimated the slope for the increase in RR relative to T_a, but even with that, the slope for HS was greater (P < 0.001) than that for Non-HS.

Progressive heat stress phase

   Ambient conditions and animal responses during the progressive heat stress phase. During this phase, T_a progressively increased from a daily mean of 20.7ºC on d 32, reaching a mean of 28.8ºC on d 36, and maintained at this high level for the following 4 days (Figure 3.11). From d 36 to 40, little night cooling occurred, with the minimum temperature reaching 22.0^{o}C at 0600 h on d 39. It has been suggested that the level of cooling at night is the most important factor in preventing problems associated with heat stress (Brown-Brandl et al., 2005a; Gaughan et al., 2000; Gaughan et al., 2007). Gaughan et al. (2000) compared daytime with nighttime cooling, and found that night cooling was more effective in lowering T_{core} of steers than daytime cooling. It also reduced overall respiration rate and rectal temperature, even though the maximum values for those parameters were higher than that of steers cooled during the day. Moreover, when beef steers were exposed to simulated cyclic temperature and heat waves, it was found the nighttime low had more influence on feed intake and total heat production than daytime high (Brown-Brandl et al., 2005a). Thus, if the T_a does not fall below a certain level at night, cows cannot dissipate enough heat to cool their bodies, and the heat will accumulate during the next day, causing heat strain (Brown-Brandl et al., 2005a). In the

current study, toward the end of the progressive heat stress phase, T_a was not increasing any further. However, T_{core} increased throughout the day, indicating an accumulation of heat in the animal's body, because temperature at night did not reach a low point to allow heat dissipation, and T_{core} continued to increase each day.

In Figure 3.11, during the day, the pattern of T_{core} cycle did not overlap with that of T_a, but was delayed by about 2 to 4 hours, depending on the day and the time. Moreover, close to the evening hours, there was a drop in T_{core} for almost every day in this phase, possibly representing the milking time, when cows were moved to the parlor. After cows were returned to the barn, T_{core} rose again, causing a second peak of the day. This pattern is usually not observed for beef steers, for which T_{core} pattern follows T_a pattern closely, peaking at about 1500 to 1800 h, and decreasing to a minimum after that (Hahn et al., 1990; Mader et al., 2009). Thus, it is likely that this pattern observed in dairy cows is due to the milking time, either because of the milking procedures per se, or because cows had the opportunity to drink water from troughs located on the way from the barn to the parlor, reducing rumen temperature, and thus T_{core}. Consumption of water at $16^{o}C$ decreases rumen temperature of beef cows by 0.5 to $3.6^{o}C$, 30 minutes after water consumption, lasting for more than 2 hours (Boehmer et al., 2009). Other authors found a decrease of about 0.3ºC of vaginal temperature of dairy cows at the milking time, even in dry cows, which were only walked through the parlor to simulate the milking (Araki et al., 1984). However, when the dry cows were not walked to the parlor, the same drop at about the same time was observed. The authors suggested that the drop may be attributed to the pre-milking wash of the udder or the shade in the parlor, since cows in that

118

experiment were housed in unshaded pens (Araki et al., 1984). It was not understood why a similar drop occurred for dry cows not walked to the parlor.

   Regression of thermal status with ambient temperature during the progressive heat stress phase. Animal response to heat stress was evaluated by linear and quadratic regression (Table 3.7). In this table, hourly increments of delays were tested, up to the point when the increase in delay decreased the value for R^2 for the specific variable. The relationship of T_{core} vs. T_a was linear for the whole day and from 1000 to 1500 h. For the data set composed of 0700, 1600 and 1900 h, the response of T_{core} to T_a was quadratic. However, the shapes of the linear and quadratic curves were similar, indicating that the linear function was as good as the quadratic. The best delay obtained was 3 hours for the whole day, and 2 hours for both the analysis from 1000 to 1500 h and the analysis at 0700, 1600 and 1900 h. However, for the later, 1 hour delay could properly express the relationship, since the R^2 values for 1 and 2 hours of delay were very similar. The T_a explained 22.1, 35.9 and 32.7% of the variation in T_{core}, for the whole day, from 1000 to 1500 h, and at 0700, 1600 and 1900 h, respectively. It is interesting that the delay for T_{core} vs. T_a was not always consistent, and it depended on the way the variable was analyzed. When the six periods were evaluated, as well as HS vs. Non-HS, the best delay for T_{core} was 4 hours for the whole day analysis (instead of 3 hours), and 0 hours for 1000 to 1500 h analysis (instead of 2 hours). Thus, the assumption of a specific delay in response of a variable relative to T_a must be made carefully, because it may vary, depending on the way the variable is recorded. Other authors found a delay of about 2 hours between the initial rise in ambient temperature and the elevation of rectal temperature when shade and sprinklers were utilized, and a delay of 5 hours when only

119

shade was utilized for dairy cows in an open housing facility with free-stalls (Igono and Johnson, 1990). For beef steers exposed to simulated cyclic temperature and heat waves, an average delay of 5.25 ± 0.25 hours, ranging from 3 to 13.5 hours, was found for the response of T_{core} (measured in the abdominal cavity with transmitters) relative to T_a (Brown-Brandl et al., 2005a). In a subsequent study, Brown-Brandl et al. (2005b) found a lag for the response of T_{core} to the change in T_a ranging from 1 to 5 hours for beef steers housed in pens and exposed to solar radiation and wind. This indicates that the delay in response varies largely, and a single lag time should not be assumed for T_{core} analysis.

In the present study, no delay in response was observed for the relationship of RR to T_a, with 26.8% of the variation in RR explained by T_a (Table 3.7). Beef steers housed in environmentally controlled chambers presented a delay in RR relative to the increase in T_a of 0 to 3 hours, averaging 2 hours (Gaughan et al., 2000). Hahn (1999) observed similar responses for beef cattle subjected to cyclical T_a in environmental chambers, with RR lagging behind T_a by 2 hour (Hahn, 1999). In contrast, when beef steers were housed in pens and exposed to solar radiation and wind, RR lagged solar radiation by only 1 hour (ranging from 0.5 to 1.25 hours), suggesting that animal's response is different whether animals are housed inside or outside (Brown-Brandl et al., 2005b).

Evaluation of T_{ear} and T_{rump} response to T_a, in the present study, showed that incorporation of 1 hour of delay only slightly changed the R^2 value compared to 0 hours (Table 3.7). So, both skin temperature relationships could be expressed without delay in response. Ambient temperature explained 35.2 and 53.1% of the variation in T_{ear} and T_{rump}, respectively. It appears T_{rump} was the variable, among all tested (i.e. T_{core}, RR, T_{ear} and T_{rump}), that better related to T_a, as it contained the highest R^2. However, other factors

120

besides T_a contributed as much as 50% to the variation in all thermal status. The relationships of RR, T_{ear} and T_{rump} to T_a were quadratic. However, the shapes of the linear and quadratic regressions were similar for all comparisons (plots not shown), indicating the linear function could be utilized without losing quality to the analysis.

Linear regressions for T_{core}, RR, T_{ear} and T_{rump} as functions of T_a are shown in Figure 3.12. The slope for T_{core} vs. T_a from 1000 to 1500 h (Figure 3.12b) was greater (P < 0.05) than that for the whole day (Figure 3.12a), so, the increase of T_{core} from 1000 to 1500 h was greater as T_a increased. Moreover, there was more variation when the whole day was analyzed, first because of the larger number of data points, and secondly, because low T_a values were included, to which T_{core} is not dependent. The slope for the relationship between T_{rump} vs. T_a (slope = 0.194°C; Figure 3.12f) was higher (P < 0.05) than the slope for T_{ear} vs. T_a (slope = 0.140°C; Figure 3.12e) indicating a greater increase in T_{rump} relative to the increase in T_a compared to T_{ear}.

In general, ambient conditions available for farmers consist of only daily maximum, minimum and mean T_a. Thus, in order to determine which parameter had the most influence on the thermal status of animals during the progressive heat stress phase, daily maximum T_{core}, RR, T_{ear} and T_{rump} were tested against daily minimum, maximum and mean T_a. The R^2 for those linear regressions are shown in Table 3.8. Mean T_a had a greater influence on maximum RR ($R^2 = 0.370$), T_{ear} ($R^2 = 0.524$), and T_{rump} ($R^2 = 0.638$) compared to minimum and maximum T_a. Minimum ($R^2 = 0.287$) or mean ($R^2 = 0.281$) T_a had about the same effect on maximum T_{core}. Thus, for the progressive heat stress phase, the mean T_a of the day could better predict the level of heat strain, as indicated by the maximum T_{core}, RR, T_{ear} and T_{rump} of cows. In agreement, Maust et al. (1972) found that

afternoon rectal temperature of dairy cows was more affected by daily mean T_a than by other ambient conditions, such as minimum and maximum T_a, RH, wind speed, and THI. Moreover, West et al. (2003) found mean T_a had more influence on afternoon milk temperature, while minimum T_a had more influence on morning milk temperature.

Regression between animal's thermal status during the progressive heat stress phase. Animal responses were compared against each other in an attempt to define any relationships (Table 3.9). The data set for these analysis were composed by the 3 hours of day when measurements were collected (at 0700, 1600, and 1900 h) for all 9 days during the progressive heat stress phase. The T_{core} lagged behind RR, T_{ear} and T_{rump} by 1 hour, with a linear response. For beef steers housed in environmentally controlled chambers and exposed to T_a cycling from 24 to 39ºC, tympanic temperature lagged behind RR by 2 hours (Gaughan et al., 2000). For the change in T_{core} as a function of T_{rump} (Figure 3.14c), when T_{rump} was lower than 32ºC and higher than 36.5ºC, the 95% CI for the linear and quadratic functions did not overlap each other; however, few data points existed below and above those values of T_{rump}, so, the quadratic function may not be reliable in the extremes, suggesting that the linear regression may be utilized without losing quality to the analysis. Respectively, RR, T_{ear} and T_{rump} explained 32.8, 25.1 and 32.2% of the variation in T_{core} during the progressive heat stress phase (Table 3.9). The slope for the regression between T_{core} vs. T_{ear} (Figure 3.13b) was not different ($P > 0.10$) from the slope of T_{core} vs. T_{rump} (Figure 3.1c) indicating the increase in T_{core} relative to the increase in T_{ear} and T_{rump} were not different.

When RR, T_{ear} and T_{rump} were related to each other, delay incorporation was not possible, since the parameters were not collected every hour, but only at 0700, 1600 and

1900 h. A slightly better fit was obtained with the quadratic regression for all the comparisons, indicated by a slightly higher R^2 (Table 3.9). However, the 95% CI of the quadratic and linear functions overlapped each other (plots not shown), suggesting the linear function could be utilized without losing quality to the analysis. Only for the relationship between T_{rump} and T_{ear} (Figure 3.13d), the 95% CI for the linear and quadratic regressions were not coincident when T_{rump} values were lower than 32°C and higher than 36.5°C, similarly to what happened for the relationship between T_{core} and T_{rump}. Because only few data points existed for that range of T_{rump}, it was suggested that the quadratic function was not reliable in those two extremes, thus, the linear could be utilized. The relationship between T_{rump} and T_{ear} was good but lower than expected; only 58% of the variation in T_{rump} was related to T_{ear}. It was speculated that the large variation within the population caused the R^2 to be low. Berman (1971) found that T_{ear} of lactating dairy cows was strongly correlated (r = 0.91, equivalent to R^2 = 0.83) to mean skin temperature, as an average of back, mid-flank, lateral abdomen, hind leg and both ears. The higher R^2 in that study could be attributed to a shorter length of study (only 4 days vs. 9 days in the present study), smaller sample size (only 4 cows vs. 14 cows in the present study), but more frequent measurements collection (8 times a day vs. 3 times in the present study).

In the current study, T_{ear} was related to 26.3% of the variation in RR, while T_{rump} was related to 30.7% (Table 3.9). No differences (P > 0.10) existed between the slopes of RR vs. T_{ear} (Figure 3.13e) and RR vs. T_{rump} (Figure 3.13f), indicating the same increase in RR relative to the increase in T_{ear} or T_{rump}. Berman (1971) found a higher correlation (r = 0.75, equivalent to R^2 = 0.56) between respiratory frequency and mean skin temperature,

as an average of back, mid-flank, lateral abdomen, hind leg and both ears. In order to represent a mean skin temperature for animals in the current study, hourly values for T_{rump} and T_{ear} were averaged and analyzed (data not shown). However, this approach did not improve the relationship of skin temperature with T_a or the relationship with other animal's thermal parameters. Spiers et al. (2004b) indicated skin temperature could dictate changes in thermoregulatory effectors, such as RR, because of the large amount of thermal receptors in the skin. However, it is difficult to identify one site throughout the animal's body that better represents the true skin temperature.

Regression of performance parameters with ambient and core body temperatures during the progressive heat stress phase. Animal performance was also tested against T_a and T_{core} (Table 3.10). This approach should help to explain how performance changes during a phase of increasing heat stress, in order to reduce heat production in the body. Since only daily values were collected for DMI and milk production, a daily minimum, maximum and mean were calculated for T_a and T_{core} in order to enable the comparisons. Linear and quadratic regressions of DMI and milk production as functions of minimum, maximum and mean T_a and T_{core} are shown in Table 3.10. Even though the R^2 for the analysis was very low, no delay existed in the response of intake to T_a or T_{core}, while milk production responded with 1 day delay. Thus, intake was affected by elevated ambient and body temperatures, and it took a certain time before it affected milk production. Spiers et al. (2004b) also found no delay in the response of DMI of dairy cows to T_{core}, but 1 day delay improved the prediction for milk production. Maust et al. (1972) indicated feed intake of dairy cows was better related to

124

the afternoon rectal temperature on the same day, while milk production exhibited a 3 day delay in response.

Dry matter intake and milk production of cows in the present study were better related to the minimum T_a or T_{core} of the day, as indicated by a higher R^2 (Table 3.10), which occurs in the early morning. This suggests that the nighttime low is even more important than the daytime peak in T_a and T_{core}. Supporting these findings, Holter et al. (1996) found that minimum daily THI was better correlated with feed intake and 4% energy-corrected milk than maximum THI, when evaluating the effects of heat stress in Jersey cows. Other researches also indicated the cooling at night may be the most important factor influencing the heat stress effects on the following day for beef steers exposed to controlled cycling T_a (Gaughan et al., 2000; Gaughan et al., 2007). In contrast, a study evaluating the effects of environmental conditions (air temperature, RH and THI) on performance of dairy cows indicated milk production was most affected by the mean THI with 2 day delay, and that DMI was most affected by mean air temperature, also with 2 day delay (West et al., 2003). In the current study, the relationship between milk production and mean T_a was also favorable, with an R^2 close to that of minimum T_a. This indicates that either minimum or mean T_a could be utilized to evaluate the effects of T_a on milk production. The minimum T_a explained 8.4% of the variation in DMI and 4.9% of the variation in milk production. Minimum T_{core} explained 3.7% of the variation in DMI and 6.5% of the variation in milk production (Table 3.10). Thus, the ambient conditions exerted more influence on feed intake, while milk production was more influenced by T_{core}. West et al. (2003) found DMI and milk production were more affected by ambient conditions than by rectal temperature. Brown-

Brandl et al. (2005a) found a stronger relationship between air temperature and feed intake for beef steers exposed to simulated cyclic T_a and heat waves. In that study, mean T_a explained 30% of the variation in average daily feed intake, as a quadratic regression (Brown-Brandl et al., 2005a), while in the current study, mean T_a explained only 6.2% of the DMI. This difference may be attributed to the use of controlled conditions by Brown-Brandl et al. (2005a), reducing the variance.

Research has shown feed intake and milk production are reduced when cows are exposed to heat stress (Berman et al., 1985; Kadzere et al., 2002). However, in this study, DMI and milk production were poorly related with T_a and T_{core} (Figure 3.14), as identified by the low R^2 for all analyses, linear or quadratic. This poor relationship may suggest that factors other than the increase in ambient and core temperatures caused a reduction in feed intake and milk production. However, from Figure 3.11, a major drop in feed intake was seen on d 36, corresponding to the beginning of the heat stress (high T_a and T_{core}). One day later (d 37), a major drop was also seen for milk production, indicating ambient and core temperatures played a role in the responses of DMI and milk production during the progressive heat stress phase. Also, the large variation within a population of animals may cause the R^2 to be very low. In a group of animals, some cows may respond more rapidly to the ambient conditions, while others respond more slowly; some animals do not respond at all. This variation in responses may impede the obtainment of conclusive results. Other authors also found a poor relationship between the depression in milk production and the increase in T_a during heat stress, as determined by small coefficients of determination ($R^2 = 0.005$) (Ravagnolo et al., 2000). These authors suggest ambient conditions explain only a small portion of the milk production

decline (Ravagnolo et al., 2000). Kendall et al. (2006) reported a low relationship between daily total milk production and maximum THI (R^2 = 0.05) for dairy cows in New Zealand. Moreover, Dïkmen and Hansen (2009) did not find a significant relationship between milk yield and rectal temperature. They suggested some cows may have a better thermoregulatory capacity, and are able to produce more milk.

Regression between performance parameters during the progressive heat stress phase. The relationship between milk production and feed intake was also analyzed. As shown in Table 3.11, milk production responded linearly to intake, with 1 day delay (Figure 3.15), so DMI on one day had a higher influence on milk production of the following day. The DMI explained 32.6% of the variation in milk production. This was lower than expected, since milk production is supposed to depend largely on feed intake. However, some authors have suggested only about 35% of the reduction in milk production during heat stress periods is due to the reduction in feed intake (Rhoads et al., 2009). This low R^2 may suggest factors other than feed intake influence the decline in milk production during hot weather. Also, the large variation within the population of animals may have contributed to the low R^2 observed in these analyses.

Sensitivity to heat stress

From the analysis of progressive heat stress phase, it was noted that the R^2 was lower than expected. This could be attributed to the large variability existing among this population of animals. By evaluating each animal individually, it was observed that each animal had a response to heat stress. Some animals responded quickly to the change in T_a, while others took longer. Kendall et al. (2006) also found variability in the response of vaginal temperature to ambient conditions among dairy cows. Also, in the present

study, DMI and milk production were more related to the minimum T_a or T_{core} of the day

for some animals, and to the maximum or mean temperatures for others. Moreover, some

cows showed a high response of T_{core} to T_a, but did not respond with changes in DMI or

milk production to the increase in T_a or T_{core}. Therefore, it is difficult to identify animals

that are sensitive or non-sensitive to heat stress. Depending on the approach tested,

animals ranked differently. In this study, we attempted to identify animal sensitivity to

heat stress by assessing the relationship between T_{core} vs. T_a and DMI vs. T_{core}(min). The

five most sensitive cows, as indentified by the highest R^2 for both relationships, were

assigned to the sensitive group (S), and the five least sensitive cows, as identified by the

lowest R^2 for both relationships, were assigned to the non-sensitive group (NS). It is not

known if this is the best approach, and if it would apply to a different population of

animals. However, it appears to be the best way to separate animals in the present study.

   Performance parameters of the sensitivity groups. Performance parameters of

animals in sensitive (S) and non-sensitive (NS) groups, during the progressive heat stress

phase (d 32 to 40) is shown in Table 3.12. The overall averages for DMI and milk

production, as an average of all days during the progressive heat stress phase, were not

different (P > 0.10) between NS and S. However, when the interaction of animal

sensitivity x d was evaluated, DMI of S was lower (P < 0.01) than NS on d 39 (Figure

3.16a). A large drop in DMI of the S group occurred on d 38 and 39, while the DMI for

NS group was maintained almost constant. This indicates that sensitive animals were

more affected by the high level of T_a during those days (Figure 3.11). Although no

differences (P > 0.10) existed for milk production for the interaction sensitivity x d, S had

a greater drop in production on d 37 than NS (Figure 3.16b), as a response to the elevated T_a on d 35 and 36 (Figure 3.11).

Thermal status of the sensitivity groups. The average RR, T_{ear}, T_{rump}, and T_{core} of animals in each sensitivity group is shown in Table 3.12. No differences ($P > 0.10$) existed between NS and S for the overall averages during the progressive heat stress phase (Table 3.12) for any parameter, or for the sensitivity x d interaction for RR (Figure 3.17a), for T_{ear} (Figure 3.17b) and for T_{rump} (Figure 3.17c). Daily average of T_{core} (Figure 3.18a) indicated a tendency ($P < 0.10$) for NS having higher T_{core} than S on d 32, which consisted of thermoneutral conditions. After d 35, when heat stress conditions occurred, T_{core} for both groups became very similar, suggesting that animals less sensitive to ambient conditions maintained a more stable body temperature, from lower to higher T_a. On the other hand, sensitive animals lowered further their body temperature when T_a was low ($P < 0.10$) and had a greater increase in T_{core} when T_a rose. From Figure 3.18b, it was noted that, under thermoneutral conditions (d 32 to 34), NS had higher maximum and minimum T_{core} for most of the days compared to S. However, under heat stress conditions (d 35 to 40), S had higher maximum T_{core} and a lower minimum T_{core} for some days, especially toward the end of the phase. This indicates a greater change in body temperature during the day for sensitive animals and suggested that NS cows were able to maintain a more constant T_{core}, while S had greater fluctuation of T_{core}. The hourly average of T_{core} during the progressive heat stress phase (Figure 3.18c) showed a tendency ($P < 0.10$) for S having lower T_{core} than NS at 0700 h. Minimum T_{core} occurred at 0700 h for S, and at 1000 h for NS.

Regression of thermal status with ambient temperature for the sensitivity

groups. Regression analysis of S and NS animals was performed in order to identify the

specific thermal characteristics that separated the two groups and improve the prediction

of thermal response. The results presented in Table 3.13 correspond to S and NS animals,

as well as all animals in the study, in order to enable the visualization of whether or not

the separation of animals into the two groups improved the R^2 for the analysis. Also,

these results represent only the progressive heat stress phase (d 32 to 40), and the best

delay in response that was obtained for each variable during that phase, presented earlier.

For the comparison between slopes, the linear regression was utilized, either because the

quadratic function was not significant, or the linear and quadratic curves were similar,

indicating that the linear function could be utilized without losing quality to the analysis.

For the analyses of T_{core} vs. T_a, the S group had a higher R^2 than the NS group

(Table 3.13), indicating that T_a contributed more to the variation in T_{core} of sensitive

animals compared to non-sensitive, as expected. The separation into groups was effective

for the T_{core} response, since the R^2 was improved when only sensitive animals were

analyzed, compared to all animals together. The linear regression of T_{core} vs. T_a, for the

whole day (Figure 3.19a), 1000 to 1500 h (Figure 3.19b) and at 0700, 1600 and 1900 h

(Figure 3.19c) showed that the slope of the S group was greater ($P < 0.05$) than that of

NS. This indicates that sensitive animals had a greater increase in T_{core} relative to the

increase in T_a. Thus, the ambient conditions had a greater impact on the internal body

temperature of animals sensitive to heat stress, as expected.

Respiration rate as a function of T_a was analyzed for S and NS groups. The R^2 of

S group ($R^2 = 0.330$) was higher than that of NS group ($R^2 = 0.253$) and all animals ($R^2 =$

0.268) (Table 3.13). However, the improvement for this variable was small. In contrast, the analysis of T_{ear} and T_{rump} as functions of T_a resulted in a higher R^2 for NS animals (R^2 = 0.378 and 0.552, for T_{ear} and T_{rump}, respectively), compared to S (R^2 = 0.341 and 0.526, for T_{ear} and T_{rump}, respectively) and all animals (R^2 = 0.352 and 0.531, for T_{ear} and T_{rump}, respectively) (Table 3.13). Sensitive animals had the lowest R^2, even when compared to the analysis of all animals, although the R^2 values were very close. However, no differences ($P > 0.10$) existed between the slopes of S and NS animals for the relationships of RR, T_{ear} and T_{rump} to T_a (Figures 3.20a, b and c, respectively). These findings indicate that the animals that responded to the increase in T_a with an elevation in T_{core}, did not exhibit similar responses for RR and skin temperatures. Thus, the lower T_{core} for NS animals was possibly associated with a lower body heat production or with the utilization of other mechanisms for heat dissipation, besides respiratory and cutaneous heat loss, which allowed them to maintain a lower T_{core}.

Regression between thermal responses for the sensitivity groups. Animal's thermal status were compared against each other to identify the responses for S and NS animals (Table 3.13). Except for the relationship of T_{rump} as a function of T_{ear}, S animals had higher R^2 compared to NS and all animals for all the comparisons (T_{core} as function of RR, T_{ear} and T_{rump}; RR as function of T_{ear} and T_{rump}). For T_{rump} vs. T_{ear}, R^2 of S (R^2 = 0.573) was lower than NS (R^2 = 0.580) and also than all animals (R^2 = 0.580); however, the R^2 values were very close. The S group had a greater ($P < 0.05$) slope compared to NS animals, for the relationship of T_{core} to RR, T_{ear} and T_{rump} (Figures 3.21a, b and c, respectively), indicating a greater increase in T_{core} of sensitive animals relative to the

increase in the other thermal status. So, animals that were sensitive for T_{core} to ambient conditions were also sensitive in other thermal responses.

Regression of performance parameters with ambient and core body temperatures for the sensitivity groups. The influence of T_a and T_{core} on DMI and milk production of NS and S animals, during the progressive heat stress phase, was evaluated by utilizing minimum daily values for T_a and T_{core}. Minimum values were utilized because they yielded the highest R^2 for the relationships, compared to daily maximum or mean, for the analysis during the progressive heat stress phase (Table 3.10). For the analysis of DMI vs. T_a(min) and DMI vs. T_{core}(min) (Table 3.13), animals in the S group had a higher R^2 ($R^2 = 0.210$ and 0.132, respectively) compared to those in the NS group ($R^2 = 0.021$ and 0.007, respectively), or with all animals ($R^2 = 0.084$ and 0.037, respectively). The separation into groups was effective for the DMI relationship with T_a and T_{core}, showing that when only sensitive animals were evaluated, the R^2 was improved. The higher R^2 for DMI vs. T_{core}(min) of the S group was expected, since animals were separated into groups based on this relationship. However, the separation into groups considerably improved the R^2 for the relationship between DMI and T_a(min). Moreover, the slope for the S group tended ($P < 0.10$) to be greater than the NS group, for both comparisons (DMI vs. T_a(min), Figure 3.22a; and DMI vs. T_{core}(min), Figure 3.22b), indicating that sensitive animals had a greater decline in DMI relative to the increase in T_a(min) and T_{core}(min) compared to non-sensitive animals.

The comparison of milk production relationship to T_a(min) showed that S animals had a higher R^2 ($R^2 = 0.130$) than NS ($R^2 = 0.015$) or all animals ($R^2 = 0.049$). However, when milk was related to T_{core}(min), the opposite was observed, and the NS group had

132

the highest R^2 value ($R^2 = 0.130$) (Table 3.13). Moreover, the slopes for milk production

vs. T_a(min) (Figure 3.22c) and milk production vs. T_{core}(min) (Figure 3.22d) were not

different ($P < 0.10$) between S and NS groups. This suggests animals that responded to

the increase in T_a and T_{core} with a reduction in DMI (i.e. S animals), exhibited the same

level of reduction in milk production, as compared to animals that did not reduce the

DMI in response to the increase in T_a and T_{core} (i.e. NS animals).

Thus, it was noted that the separation into groups based on the sensitivity of

animals improved the quality of the analysis, but only for some variables. Also, it was

expected by separating animals in the two groups, the R^2 would be considerably

increased, since it was speculated that the low R^2 was a result of the great variability

within a population of animals. However, for most cases, there was only a slight increase

in the R^2, indicating that many other factors affected the parameters, besides the specific

independent variable tested, during a progressive heat stress phase.

CONCLUSIONS

Heat stress in the present study resulted in the classical responses of reduced feed

intake and milk production, and elevated respiration rate, surface temperature and

internal body temperature of dairy cows. Even short exposures to heat stress (i.e. period

4) altered cow performance and thermal status. Parameters measured during a progressive

heat stress phase exhibited different delays in response to changes in thermal status and

ambient temperature. Core body temperature had the most varying delay in response of

all parameters evaluated, ranging from 0 to 4 hours of delay. The other thermal

parameters (respiration rate, ear and rump skin temperatures) and also dry matter intake

responded to the increase in ambient temperature without any delay. Milk production responded to the decline in intake, and also to the increase in ambient temperature with one day of delay. Daily minimum ambient and core temperatures were better predictors of the decline in intake and milk production during a phase of progressive heat stress, while daily mean T_a had greater influence on the maximum RR, skin and core body temperatures.

Animals in the present study differed in their response to heat stress, either by utilizing different mechanisms to cope or by not responding to it. This emphasizes the importance of utilizing more than one parameter to separate animals based on the sensitivity to heat stress. If the most sensitive animals were identified within the group, the onset of heat stress could be more easily determined. Thus, preventive actions could be taken to alleviate the negative impact of heat stress on production and well-being of the whole herd.

Table 3.1. Composition of the total mixed ration

Feed ingredient	% of DM
Alfalfa hay	13.8
Alfalfa silage	13.8
Corn silage	18.1
Shelled corn	20.74
Soybean meal, 48%	3.8
Whole cottonseed	4.8
Roasted soybeans	4.0
Wet brewer's grains	7.2
Soybean hulls	11.7
Dicalcium phosphate	0.51
Limestone	0.62
Sodium bicarbonate	0.44
TM salt	0.26
Vitamin ADE premix #1	0.09
Vitamin E premix	0.09
RTM trace mineral	0.05
Chemical Analysis	
Dry matter	52.1
Crude protein	18.1
NDF	42.2
ADF	26.8

Figure 3.1. Average core body temperature (T$_{core}$) of all animals throughout the day for the entire 63 days of study. Showing milking and feeding periods, and the longest and most sustained rise in T$_{core}$ during the day (from 1000 to 1500 h).

Table 3.2. Two days representing each of the six periods, ambient conditions, and Non-HS vs. HS designation

Period	Days	Condition	Non-HS vs. HS
1	15 and 16	Thermoneutral	
2	18 and 19	Thermoneutral	Non-HS
3	32 and 33	Thermoneutral	
4	36 and 37	Heat stress	
5	39 and 40	Heat stress	HS
6	48 and 49	Heat stress	
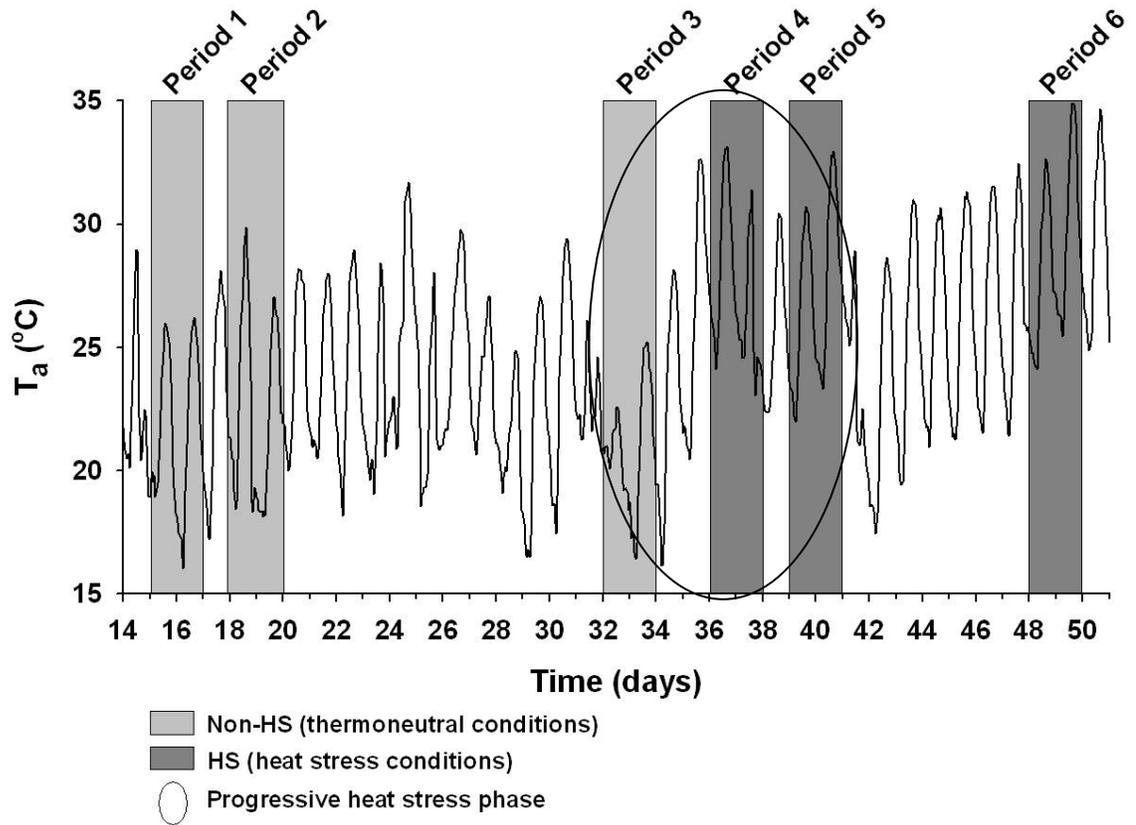
Figure 3.2. Ambient temperature (T_a) during the study, showing the six periods (thermoneutral or heat stress) and the progressive heat stress phase (d 32 to 40).
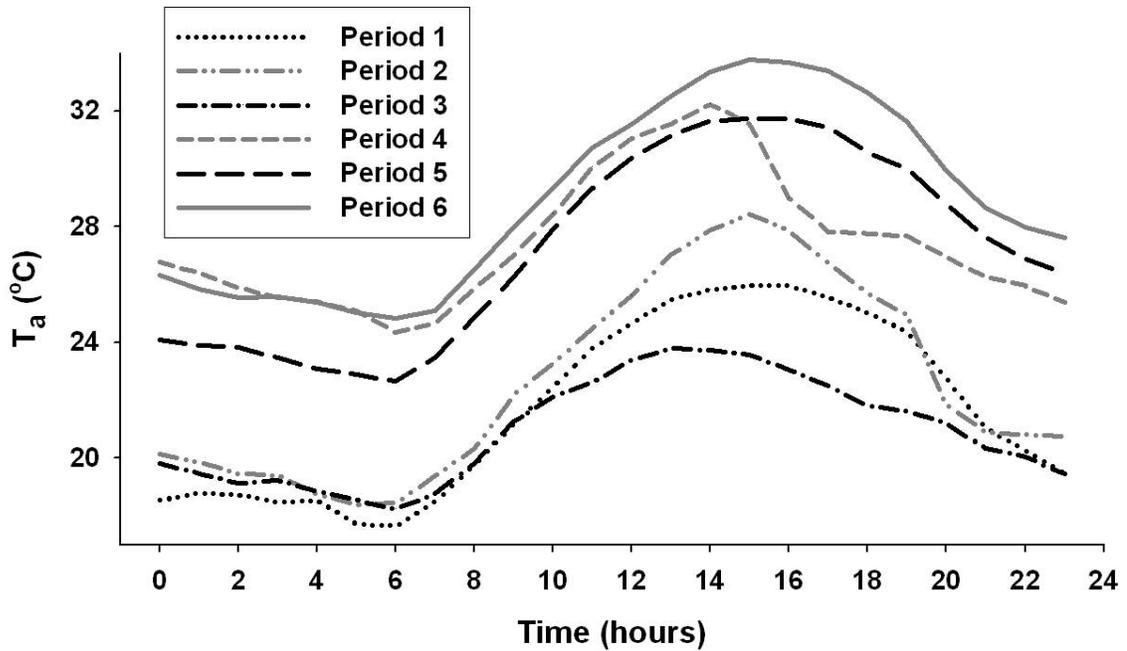
Figure 3.3. Ambient temperature (T_a) as a function of time (hours of day) for each of the six periods. Values consisted of the average by hour for the two consecutive days that represented each period.

Table 3.3. Average ambient temperature (T_a), DIM, DMI, milk production, respiration rate (RR), ear skin temperature (T_{ear}), rump skin temperature (T_{rump}) and core body temperature (T_{core}) for each of the six periods

Item	Periods						SE_{pooled}	P value
	1	2	3	4	5	6		
Ambient conditions[1]								
T_a(mean), °C	21.65 [e]	22.57 [d]	20.96 [f]	27.46 [b]	27.25 [c]	28.97 [a]	0.03	< 0.0001
T_a(min), °C	17.16 [f]	18.22 [d]	17.41 [e]	23.58 [b]	22.65 [c]	24.77 [a]	0	< 0.0001
T_a(max), °C	26.10 [e]	28.46 [d]	23.89 [f]	32.26 [b]	31.84 [c]	33.77 [a]	0	< 0.0001
Animal's parameters[2]								
DIM, d	152	155	169	173	176	185	49.80	-
DMI, kg/d	25.42 [b]	28.43 [a]	24.74 [b]	22.54 [c]	22.49 [c]	24.11 [b]	0.95	< 0.0001
Milk, kg/d	32.29 [ab]	32.77 [a]	32.02 [ab]	31.16 [bc]	30.03 [c]	30.06 [c]	1.14	< 0.0001
RR, bpm	55.13 [c]	53.58 [c]	47.34 [d]	61.05 [b]	74.79 [a]	76.46 [a]	1.95	< 0.0001
T_{ear}, °C	34.72 [d]	34.47 [e]	33.70 [f]	34.96 [c]	35.21 [b]	35.46 [a]	0.10	< 0.0001
T_{rump}, °C	34.40 [d]	34.39 [d]	33.24 [e]	34.91 [c]	35.17 [b]	35.61 [a]	0.09	< 0.0001
T_{core}, °C (whole day)	38.67 [c]	38.68 [c]	38.59 [c]	39.05 [b]	39.25 [a]	39.24 [a]	0.08	< 0.0001
T_{core}, °C (1000 to 1500 h)	38.64 [b]	38.64 [b]	38.50 [b]	39.05 [a]	39.14 [a]	39.16 [a]	0.09	< 0.0001

[a, b] Within a row, means without a common superscript differ;
[1] Values consisted of the average for the two consecutive days that represented each period;
[2] Values consisted of the average of all cows for the two consecutive days that represented each period.
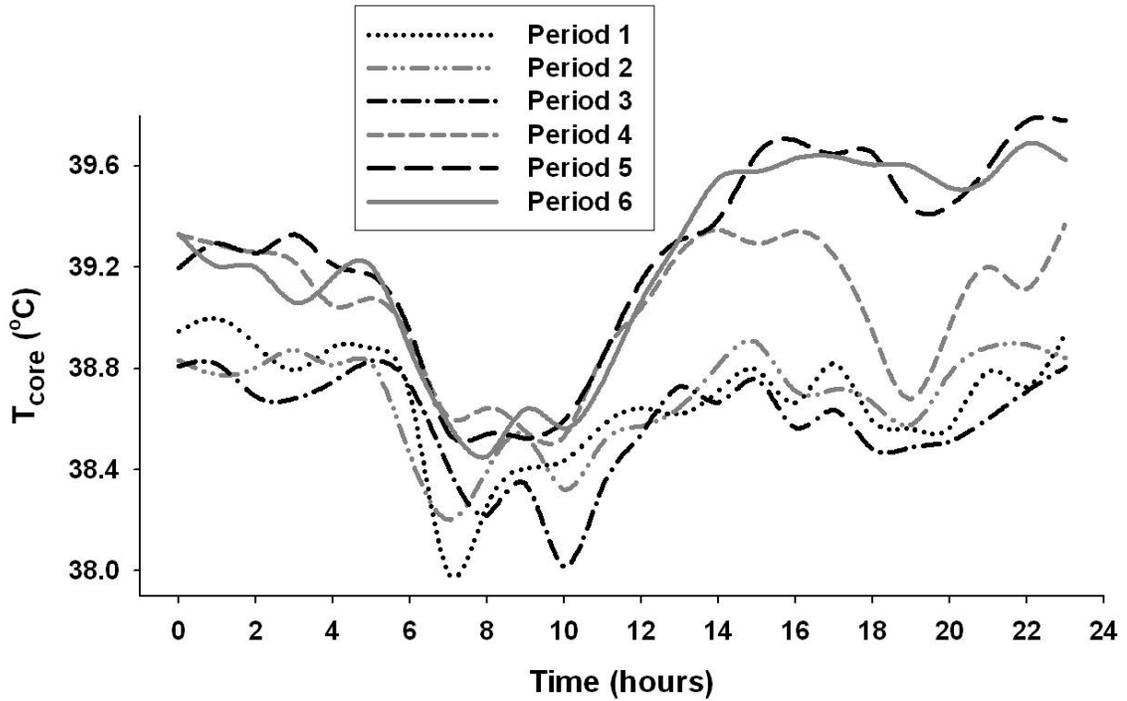
Figure 3.4. Core body temperature (T_{core}) as a function of time (hours of day) for each of the six periods. Values consisted of the average of all cows by hour for the two consecutive days that represented each period.

Table 3.4. Coefficients of determination (R^2) for the linear and quadratic regressions of core body temperature (T_{core}) as a function of ambient temperature (T_a) with 0 to 5 hours delay in response for the whole day, and 0 and 1 hour delay in response from 1000 to 1500 h, for each of the six periods

142

| Regression	Delay in response (h)		Linear Periods 1	2	3	4	5	6		Quadratic Periods 1	2	3	4	5	6
T_{core} vs. T_a (whole day[1])	0	R^2	0.003	0	0.009	0.013	0.046	0.073	R^2	0.019	0.031	0.019	0.021	0.052	0.074
		P	> 0.05	> 0.05	< 0.05	< 0.05	< 0.05	< 0.05	P	< 0.05	< 0.05	> 0.05	> 0.05	> 0.05	> 0.05
	1	R^2	0.006	0	0.001	0.039	0.048	0.131	R^2	0.020	0.011	0.009	0.039	0.098	0.131
		P	> 0.05	> 0.05	> 0.05	< 0.05	< 0.05	< 0.05	P	< 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05
	2	R^2	0.001	0.001	0	0.063	0.170	0.184	R^2	0.002	0.002	0	0.069	0.178	0.190
		P	> 0.05	> 0.05	> 0.05	< 0.05	< 0.05	< 0.05	P	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05
	3	R^2	0.004	0.005	0.004	0.045	0.232	0.252	R^2	0.043	0.035	0.037	0.073	0.283	0.289
		P	> 0.05	> 0.05	> 0.05	< 0.05	< 0.05	< 0.05	P	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
	4	R^2	0.014	0.005	0.002	0.010	0.249	0.250	R^2	0.090	0.044	0.081	0.096	0.347	0.319
		P	< 0.05	> 0.05	> 0.05	> 0.05	< 0.05	< 0.05	P	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
	5	R^2	0.027	0.010	0.002	0.005	0.248	0.222	R^2	0.092	0.042	0.055	0.085	0.347	0.298
		P	< 0.05	> 0.05	> 0.05	> 0.05	< 0.05	< 0.05	P	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
T_{core} vs. T_a (1000 to 1500 h)	0	R^2	0.057	0.113	0.266	0.233	0.395	0.464	R^2	0.057	0.129	0.268	0.236	0.398	0.465
		P	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	P	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05
	1	R^2	0.016	0.029	0.084	0.080	0.302	0.317	R^2	0.016	0.049	0.101	0.093	0.305	0.329
		P	> 0.05	> 0.05	< 0.05	< 0.05	< 0.05	< 0.05	P	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05

[1] Hours representing the milking times (0500 to 0700 and 1700 to 1900 h) were removed from the data set;
Data set for regression analysis consisted of the average by hour, for each cow, of the two consecutive days that represented each period; regression curves were fitted for all those data points obtained, without further averaging;
Highlighted cells indicate the best delay in response;
The P value below each R^2 value represents the significance of the linear or quadratic regressions.
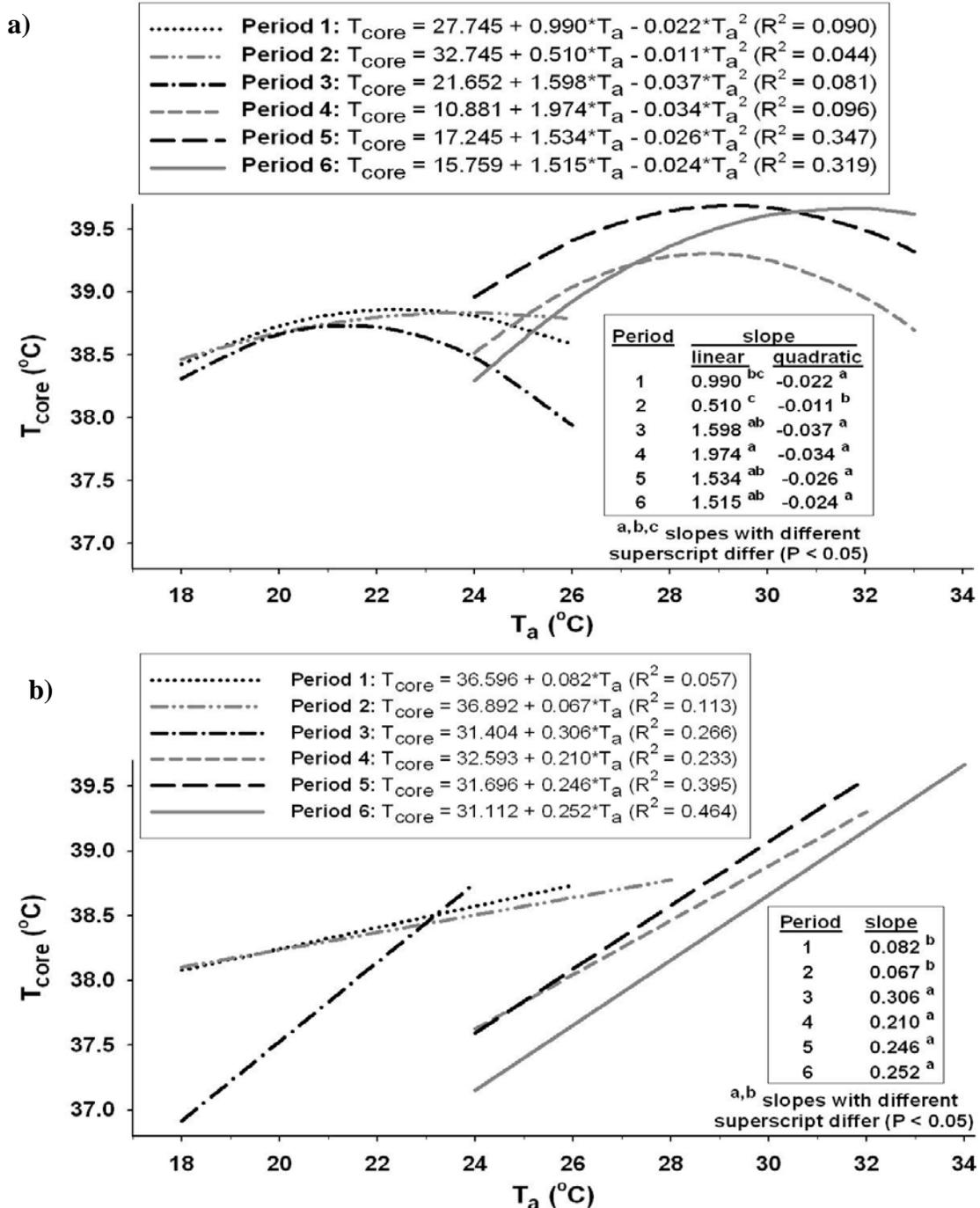
Figure 3.5. Regression of core body temperature (T$_{core}$) as a function of ambient temperature (T$_a$), with the best delay in response for the relationship, for each of the six periods. Figure a shows the quadratic regression of T$_{core}$ (for the whole day) vs. T$_a$ with 4 hours delay. Figure b shows the linear regression of T$_{core}$ (from 1000 to 1500 h) vs. T$_a$ with no delay in response. The range of T$_a$ values varies among periods; no extrapolation was utilized for curves. Data set for regression analysis consisted of the average by hour, for each cow, of the two consecutive days that represented each period; regression curves were fitted for all those data points obtained, without further averaging.
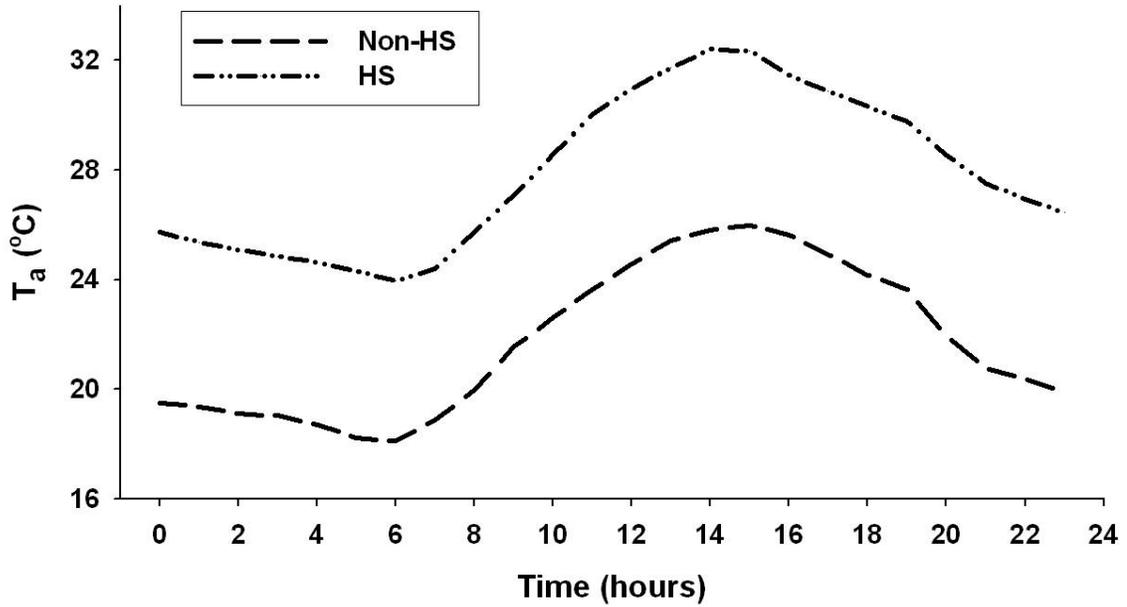
Figure 3.6. Ambient temperature (T_a) as a function of time (hours of day) for Non-HS and HS conditions. Values for Non-HS consisted of the average by hour of periods 1, 2, and 3; values for HS consisted of the average by hour of periods 4, 5 and 6.

Table 3.5. Average ambient temperature (T_a), DIM, DMI, milk production, respiration rate (RR), ear skin temperature (T_{ear}), rump skin temperature (T_{rump}) and core body temperature (T_{core}) for Non-HS and HS conditions

Item	Non-HS	HS	SE$_{pooled}$	P value
Ambient conditions[1]				
T_a(mean), ^{o}C	21.75	27.88	0.04	< 0.0001
T_a(min), ^{o}C	17.59	23.67	0.10	< 0.0001
T_a(max), ^{o}C	26.15	32.62	0.22	< 0.0001
Animal's parameters[2]				
DMI, kg/d	26.26	23.06	0.85	< 0.0001
Milk, kg/d	32.37	30.42	1.35	< 0.0001
RR, bpm	52.09	70.86	1.75	< 0.0001
T_{ear}, ^{o}C	34.30	35.21	0.08	< 0.0001
T_{rump}, ^{o}C	34.03	35.24	0.07	< 0.0001
T_{core}, ^{o}C (whole day)	38.65	39.18	0.07	< 0.0001
T_{core}, ^{o}C (1000 to 1500h)	38.59	39.12	0.08	< 0.0001

[1] Values for Non-HS consisted of the average of periods 1, 2, and 3; values for HS consisted of the average of periods 4, 5, and 6;
[2] Values for Non-HS consisted of the average of all cows in periods 1, 2, and 3; values for HS consisted of the average of all cows in periods 4, 5, and 6.
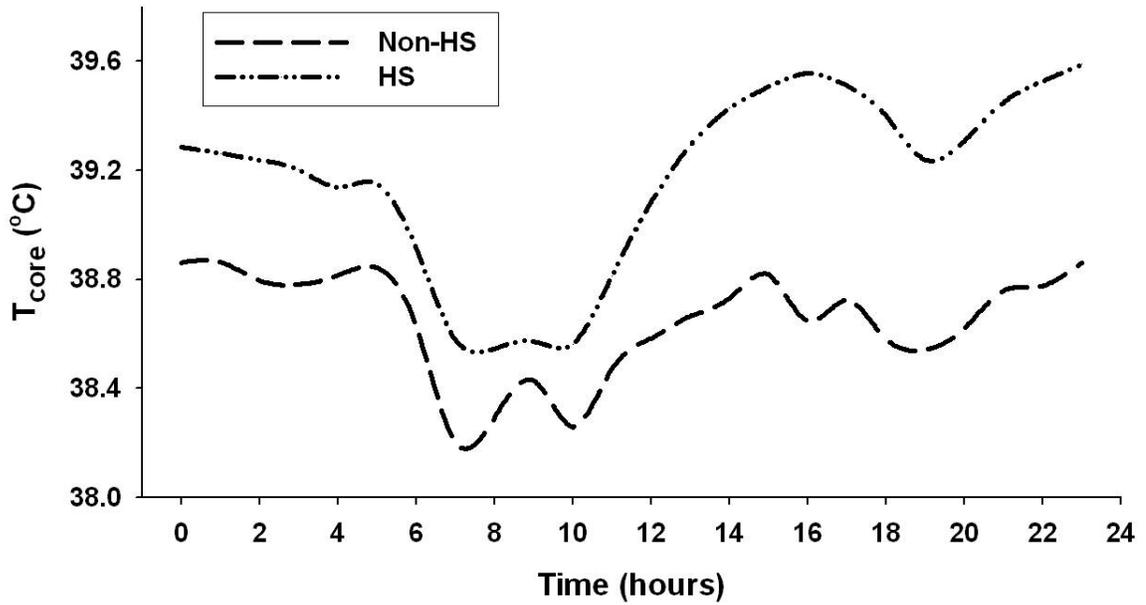
Figure 3.7. Average core body temperature (T_{core}) as a function of time (hours of day) for Non-HS and HS conditions. Values for Non-HS consisted of the average by hour of all cows in periods 1, 2, and 3; values for HS consisted of the average by hour of all cows in periods 4, 5 and 6.
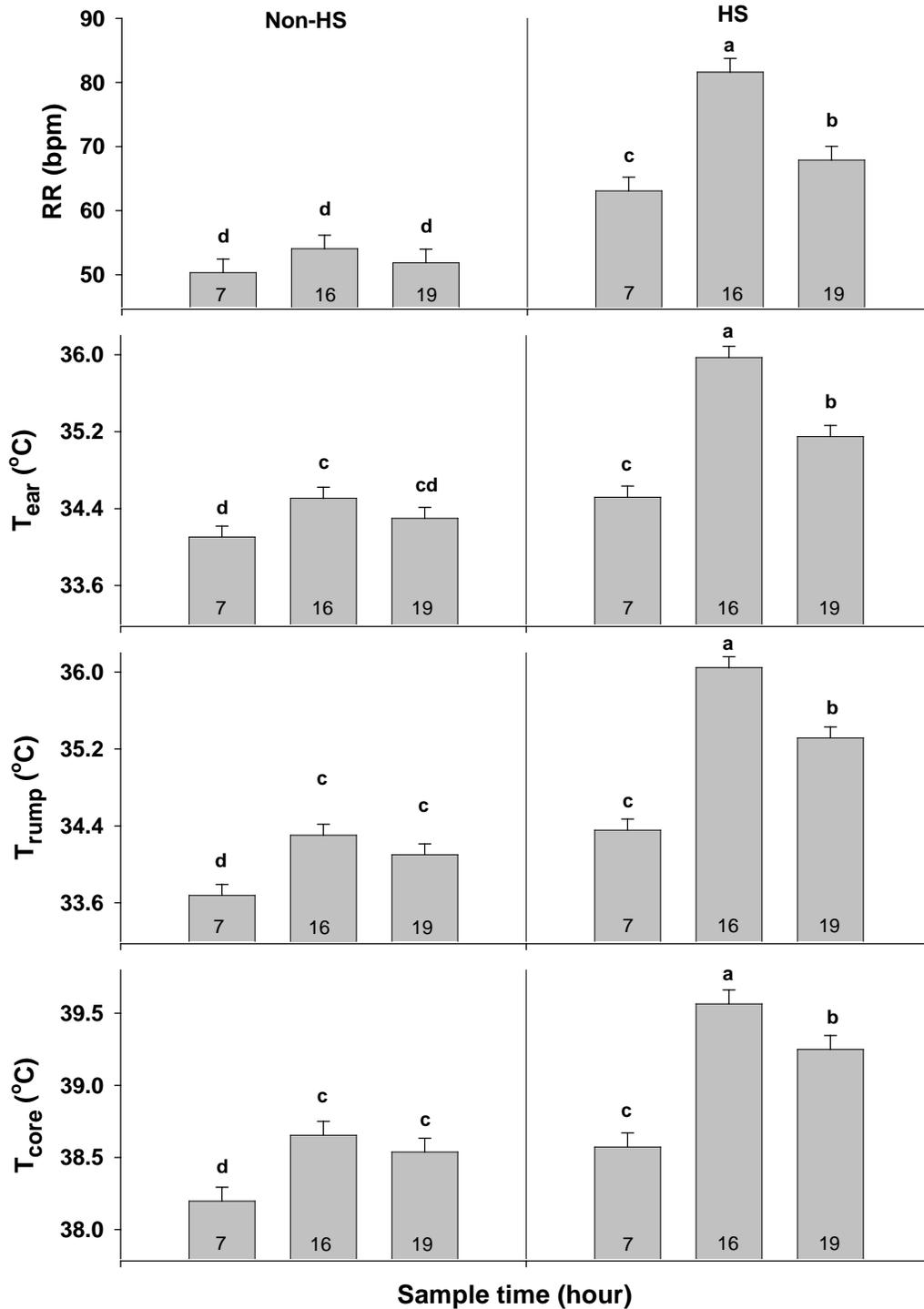
Figure 3.8. Average respiration rate (RR), ear (T$_{ear}$) and rump skin temperatures (T$_{rump}$), and core body temperature (T$_{core}$) for all cows as a function of time (hour of day), for each condition (Non-HS vs. HS). The same letter over bars indicates those that are not significantly different (P < 0.05) within a dependent variable. The vertical line on top of each variable bar is 1 SEM. Values for Non-HS consisted of the average by hour of all cows in periods 1, 2, and 3; values for HS consisted of the average by hour of all cows in periods 4, 5 and 6.

Table 3.6. Coefficients of determination (R^2) for the linear and quadratic regressions of core body temperature (T_{core}) for the whole day and from 1000 to 1500 h, respiration rate (RR), ear (T_{ear}) and rump skin temperatures (T_{rump}) as a function of ambient temperature (T_a) with delay in response ranging from 0 to 5 hours, for the Non-HS and HS conditions

| Regression	Delay in response (h)		Non-HS Linear	Non-HS Quadratic	HS Linear	HS Quadratic
T_{core} vs. T_a (whole day[1])	0	R^2	0.001	0.023	0.043	0.051
		P	> 0.05	< 0.05	< 0.05	< 0.05
	1	R^2	0	0.015	0.085	0.088
		P	> 0.05	< 0.05	< 0.05	> 0.05
	2	R^2	0.001	0.001	0.132	0.133
		P	> 0.05	> 0.05	< 0.05	> 0.05
	3	R^2	0.006	0.020	0.164	0.174
		P	< 0.05	< 0.05	< 0.05	< 0.05
	4	R^2	0.010	0.035	0.150	0.176
		P	< 0.05	< 0.05	< 0.05	< 0.05
	5	R^2	0.018	0.038	0.136	0.171
		P	< 0.05	< 0.05	< 0.05	< 0.05
T_{core} vs. T_a (1000 to 1500 h)	0	R^2	0.102	0.107	0.299	0.300
		P	< 0.05	> 0.05	< 0.05	> 0.05
	1	R^2	0.040	0.040	0.186	0.188
		P	< 0.05	> 0.05	< 0.05	> 0.05
RR vs. T_a	0	R^2	0.078	0.139	0.373	0.463
		P	< 0.05	< 0.05	< 0.05	< 0.05
	1	R^2	0.064	0.132	0.305	0.409
		P	< 0.05	< 0.05	< 0.05	< 0.05
T_{ear} vs. T_a	0	R^2	0.130	0.178	0.625	0.653
		P	< 0.05	< 0.05	< 0.05	< 0.05
	1	R^2	0.109	0.174	0.620	0.697
		P	< 0.05	< 0.05	< 0.05	< 0.05
T_{rump} vs. T_a	0	R^2	0.265	0.308	0.746	0.753
		P	< 0.05	< 0.05	< 0.05	< 0.05
	1	R^2	0.235	0.309	0.738	0.775
		P	< 0.05	< 0.05	< 0.05	< 0.05

[1] Hours representing the milking times (0500 to 0700 and 1700 to 1900 h) were removed from the data set; Data set for regression analysis consisted of all data points obtained for periods 1, 2, and 3, for Non-HS, and for periods 4, 5, and 6, for HS, without averaging;
Highlighted cells indicate the best delay in response;
The P value below each R^2 value represents the significance of the linear or quadratic regressions.
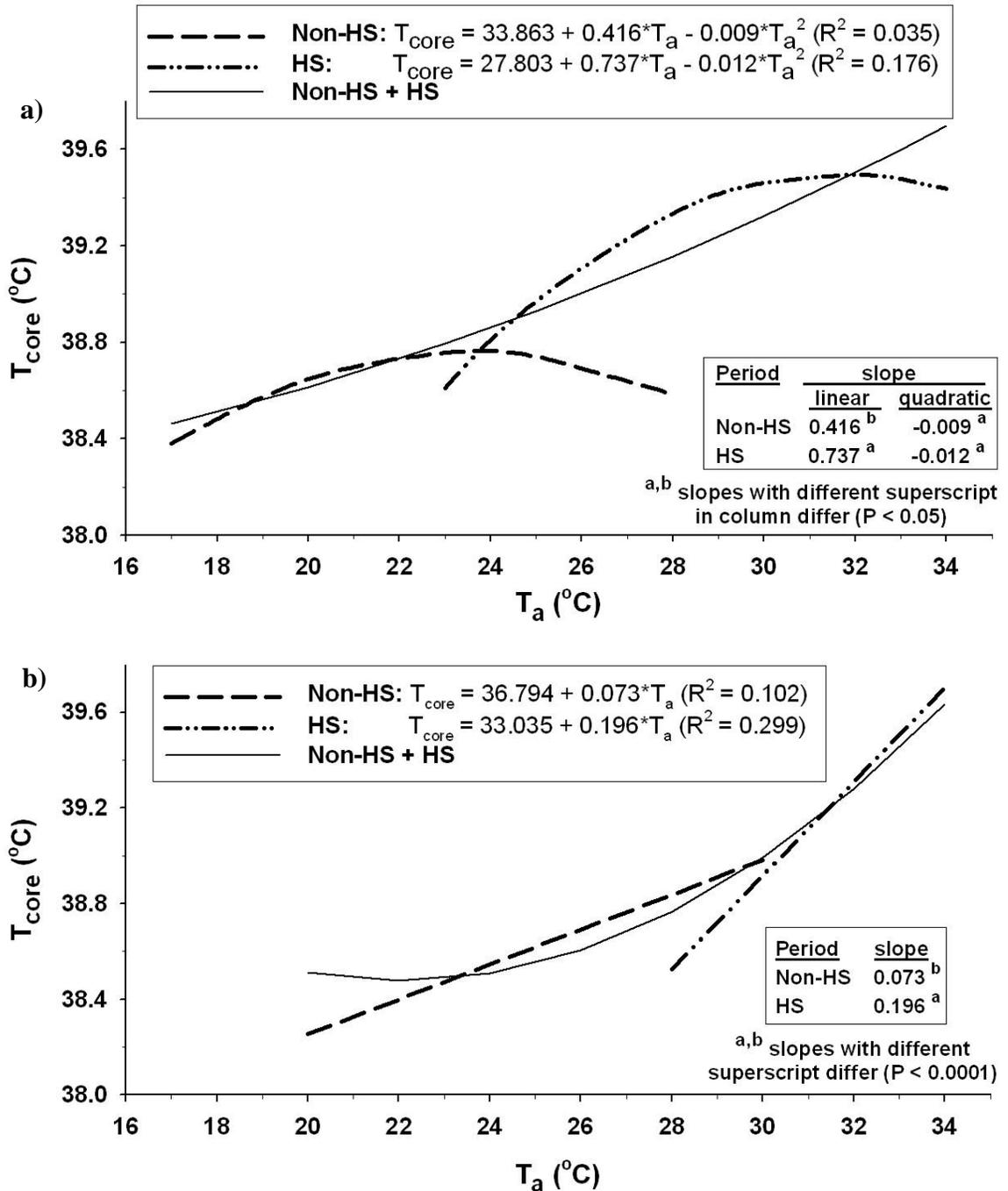
Figure 3.9. Regression of core body temperature (T_{core}) as a function of ambient temperature (T_a), with the best delay in response for the relationship, for Non-HS and HS. Figure a shows the quadratic regression of T_{core} (for the whole day) vs. T_a with 4 hours delay. Figure b shows the linear regression of T_{core} (from 1000 to 1500 h) vs. T_a with no delay in response. A quadratic regression combining both Non-HS and HS conditions is also shown for each graph. The range of T_a values varies between conditions; no extrapolation was utilized for curves. Data set for regression analysis consisted of all data points obtained for periods 1, 2, and 3, for Non-HS, and for periods 4, 5, and 6, for HS, without averaging.
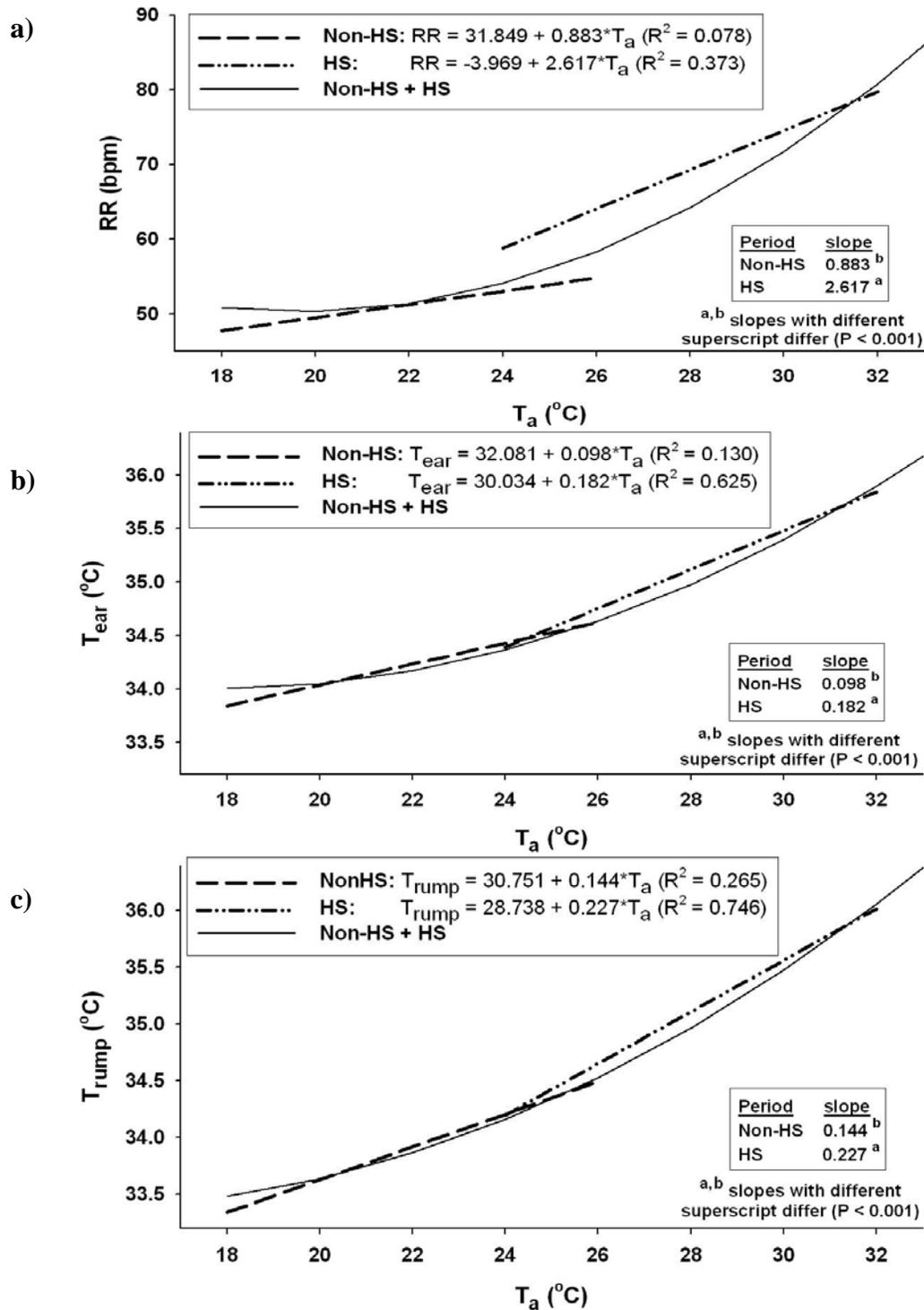
Figure 3.10. Linear regression of thermal status as a function of ambient temperature (T_a), with no delay in response, for Non-HS and HS. Figures a, b and c show respiration rate (RR), ear (T_{ear}) and rump skin temperatures (T_{rump}) vs. T_a, respectively. A quadratic regression combining both Non-HS and HS conditions is also shown for each graph. The range of T_a values varies between conditions; no extrapolation was utilized for curves. Data set for regression analysis consisted of all data points obtained for periods 1, 2, and 3, for Non-HS, and for periods 4, 5, and 6, for HS, without averaging.

150
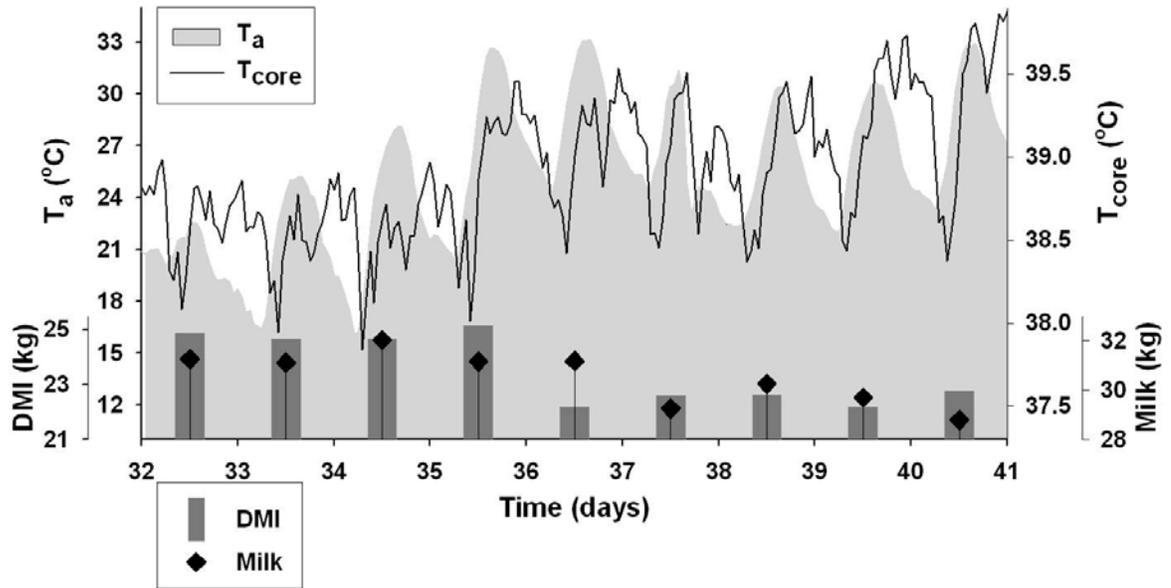
Figure 3.11. Summary of ambient temperature (T_a) and animals responses (core body temperature (T_{core}), DMI and milk production) during a phase of progressive heat stress (d 32 to 40).

Table 3.7. Coefficients of determination (R^2) for the linear and quadratic regressions of core body temperature (T_{core}, for the whole day, from 1000 to 1500 h, and at 0700, 1600 and 1900 h), respiration rate (RR), ear (T_{ear}) and rump skin temperatures (T_{rump}) as a function of ambient temperature (T_a) with delay in response ranging from 0 to 4 hours, during the progressive heat stress phase (d 32 to 40)

Delay in response (h)		T_{core} vs. T_a (whole day[1])		T_{core} vs. T_a (1000 to 1500 h)		T_{core} vs. T_a (0700, 1600 and 1900 h)		RR vs. T_a		T_{ear} vs. T_a		T_{rump} vs. T_a	
		L[2]	Q[3]	L	Q	L	Q	L	Q	L	Q	L	Q
0	R^2	0.127	0.128	0.284	0.306	0.287	0.293	0.268	0.281	0.352	0.380	0.531	0.534
	P	< 0.05	> 0.05	< 0.05	< 0.05	< 0.05	> 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	> 0.05
1	R^2	0.175	0.176	0.333	0.339	0.327	0.338	0.250	0.280	0.342	0.387	0.526	0.541
	P	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
2	R^2	0.204	0.205	0.359	0.359	0.330	0.340	0.239	0.268	0.330	0.373	0.511	0.528
	P	< 0.05	> 0.05	< 0.05	> 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
3	R^2	0.221	0.222	0.359	0.360	0.313	0.319	-	-	-	-	-	-
	P	< 0.05	> 0.05	< 0.05	> 0.05	< 0.05	> 0.05						
4	R^2	0.208	0.210	0.335	0.336	-	-	-	-	-	-	-	-
	P	< 0.05	< 0.05	< 0.05	> 0.05								

[1] Hours representing the milking times (0500 to 0700 and 1700 to 1900 h) were removed from the data set;
[2] Linear regression;
[3] Quadratic regression;
Data set for regression analysis consisted of hourly values of all 9 days during the progressive heat stress phase, for all cows, without averaging;
Highlighted cells indicate the best delay in response;
The P value below each R^2 value represents the significance of the linear or quadratic regressions;
For each variable, a decline in R^2 from one delay to the next indicates that test for subsequent delays were not necessary, and thus, cells were left empty.
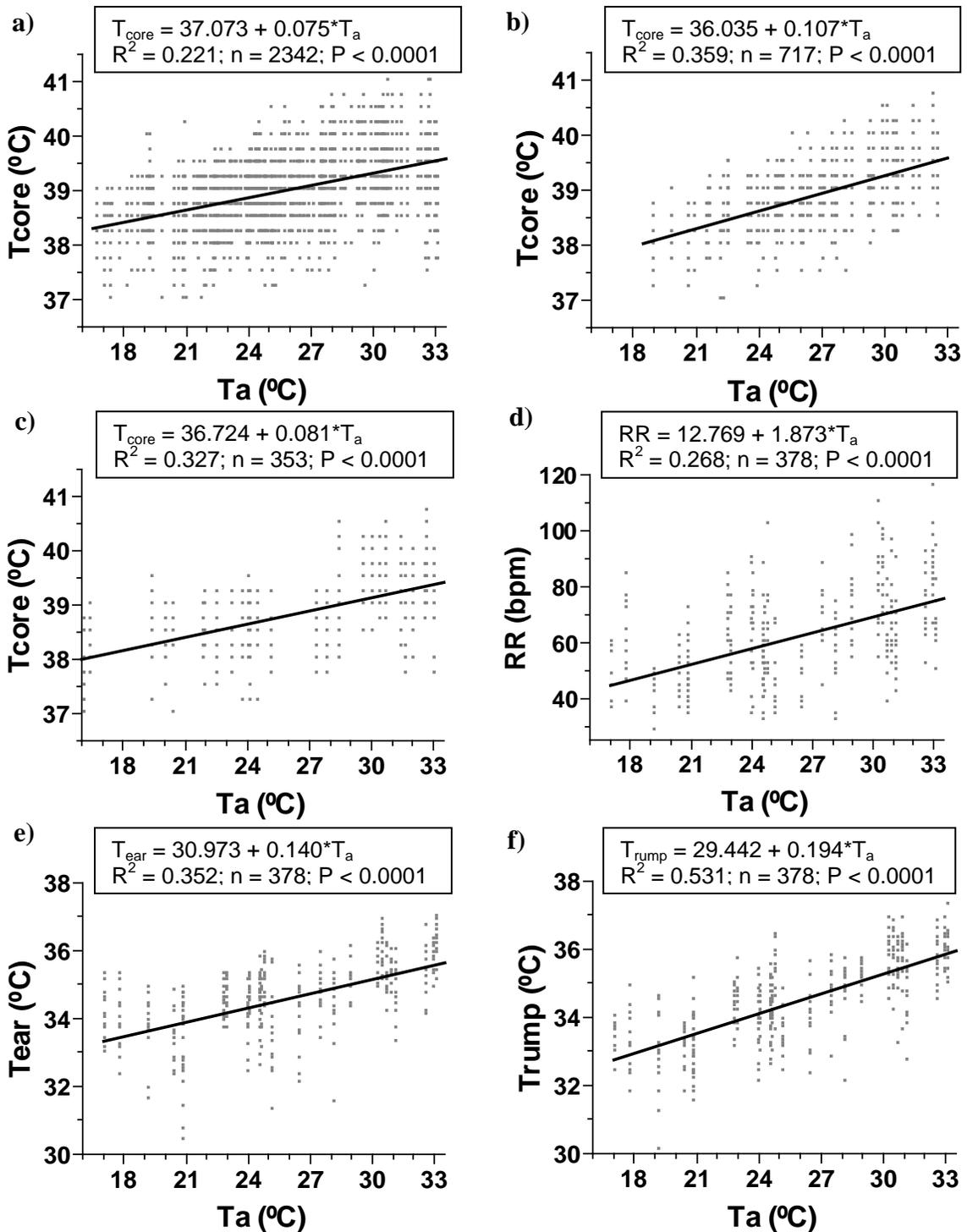
Figure 3.12. Linear regressions of thermal status as a function of ambient temperature (T_a), with the best delay for the relationship, during the progressive heat stress phase (d 32 to 40). They include: a) T_{core} (whole day) vs. T_a with 3 hours delay; b) T_{core} (from 1000 to 1500 h) vs. T_a with 2 hours delay; c) T_{core} (at 0700, 1600 and 1900 h) vs. T_a with 1 hour delay; d) RR vs. T_a with no delay; e) T_{ear} vs. T_a with no delay; f) T_{rump} vs. T_a with no delay. Each point represents hourly values for all 9 days during the phase, for each individual animal. Information in the box refers to characteristics for linear regression through the points.

153

Table 3.8. Coefficients of determination (R^2) for the linear regressions of daily maximum core body temperature (T_{core}), respiration rate (RR), ear (T_{ear}) and rump skin temperatures (T_{rump}) as functions of daily minimum, maximum and mean ambient temperature (T_a), during the progressive heat stress phase (d 32 to 40)

Regression		T_a (min)	T_a (max)	T_a (mean)
T_{core} (max)	R^2	0.287	0.231	0.281
	P	< 0.05	< 0.05	< 0.05
RR (max)	R^2	0.239	0.321	0.370
	P	< 0.05	< 0.05	< 0.05
T_{ear} (max)	R^2	0.356	0.445	0.524
	P	< 0.05	< 0.05	< 0.05
T_{rump} (max)	R^2	0.430	0.542	0.638
	P	< 0.05	< 0.05	< 0.05

Data set for regression analysis consisted of daily maximum, minimum and mean of all 9 days during the progressive heat stress phase, for all cows;
Highlighted cells indicate the best response;
The P value below each R^2 value represents the significance of the linear regression.

Table 3.9. Coefficients of determination (R^2) for the linear and quadratic regressions between animals thermal status (core body temperature (T_{core}), respiration rate (RR), ear (T_{ear}) and rump skin temperatures (T_{rump})), with 0 to 2 hours delay in response, during the progressive heat stress phase (d 32 to 40)

Delay in response (h)		T_{core}[1] vs. RR		T_{core}[1] vs. T_{ear}		T_{core}[1] vs. T_{rump}		T_{rump} vs. T_{ear}[2]		RR vs. T_{ear}[2]		RR vs. T_{rump}[2]	
		L[3]	Q[4]	L	Q	L	Q	L	Q	L	Q	L	Q
0	R^2	0.291	0.302	0.187	0.208	0.274	0.314	0.580	0.601	0.263	0.275	0.307	0.319
	P	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
1	R^2	0.328	0.333	0.251	0.274	0.322	0.371	-	-	-	-	-	-
	P	< 0.05	> 0.05	< 0.05	< 0.05	< 0.05	< 0.05						
2	R^2	0.210	0.232	0.157	0.212	0.235	0.279	-	-	-	-	-	-
	P	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05						

[1] T_{core} represented by 0700, 1600 and 1900 h only;
[2] Delay in response was not possible because parameters were not measured every hour;
[3] Linear regression;
[4] Quadratic regression;
Data set for regression analysis consisted of hourly values of all 9 days during the progressive heat stress phase, for all cows, without averaging;
Highlighted cells indicate the best delay in response;
The P value below each R^2 value represents the significance of the linear or quadratic regressions.
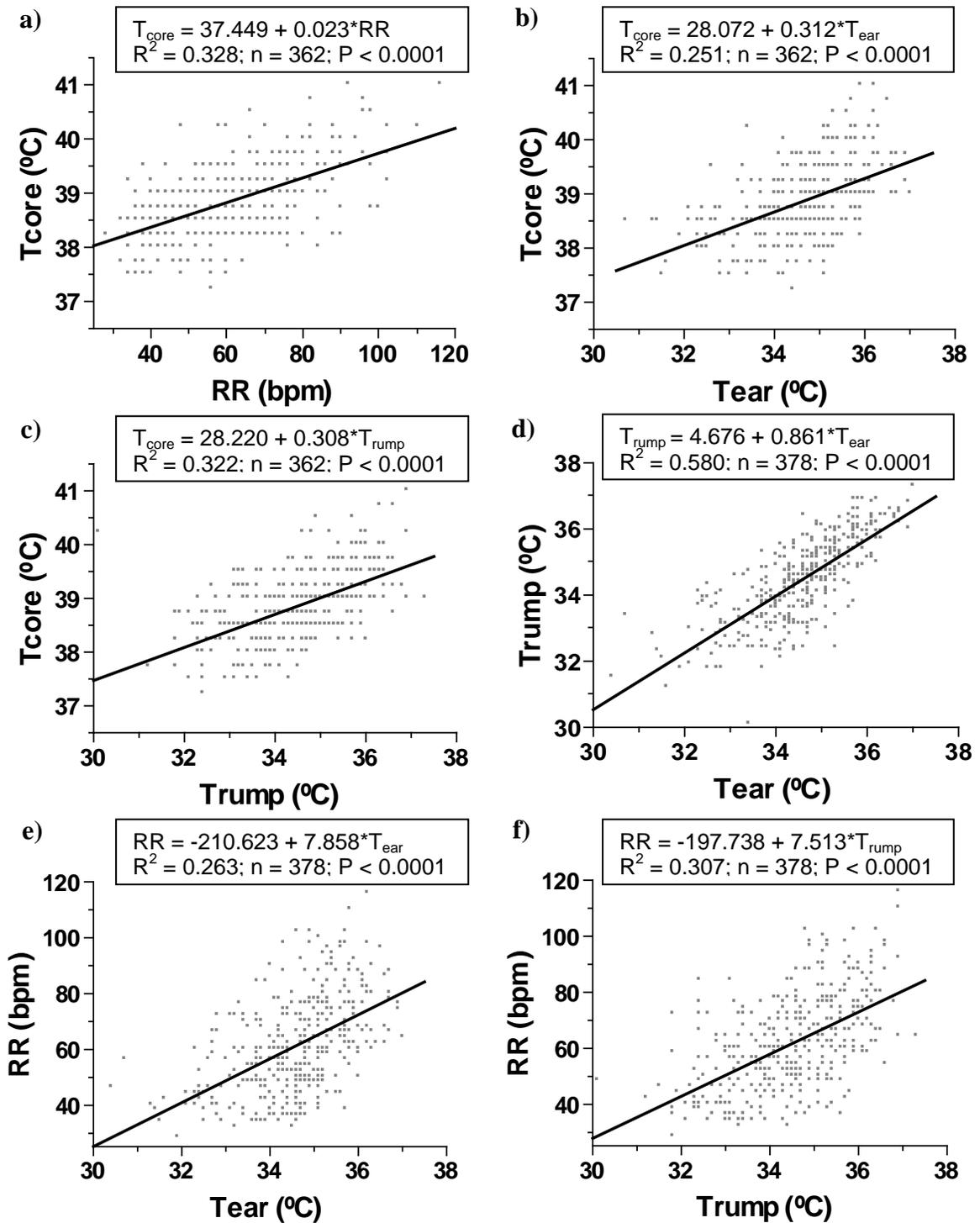
Figure 3.13. Linear regressions between thermal status, with the best delay in response for the relationship, during the progressive heat stress phase (d 32 to 40). They include: a) T_{core} vs. RR with 1 hour delay; b) T_{core} vs. T_{ear} with 1 hour delay; c) T_{core} vs. T_{rump} with 1 hour delay; d) T_{rump} vs. T_{ear} with no delay; e) RR vs. T_{ear} with no delay; f) RR vs. T_{rump} with no delay. T_{core} consisted of values at 0700, 1600 and 1900 h. Each point represents hourly values for all 9 days during the phase, for each individual animal. Information in the box refers to characteristics for linear regression through the points.

Table 3.10. Coefficients of determination (R^2) for the linear and quadratic regressions of DMI and milk production as functions of ambient temperature (T_a) minimum, maximum or mean daily, and core body temperature (T_{core}) minimum, maximum or mean daily, with delay in response ranging from 0 to 2 hours, during the progressive heat stress phase (d 32 to 40)

Delay in response (d)		Linear — T_a			Quadratic — T_a				Linear — T_{core}			Quadratic — T_{core}		
DMI vs.		min	max	mean	min	max	mean		min	max	mean	min	max	mean
0	R^2	0.084	0.043	0.062	0.091	0.044	0.062	R^2	0.037	0.003	0.017	0.038	0.012	0.025
	P	< 0.05	< 0.05	< 0.05	> 0.05	> 0.05	> 0.05	P	< 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05
1	R^2	0.064	0.049	0.055	0.074	0.049	0.064	R^2	0.012	0.001	0.001	0.015	0.039	0.046
	P	< 0.05	< 0.05	< 0.05	> 0.05	> 0.05	> 0.05	P	> 0.05	> 0.05	> 0.05	> 0.05	< 0.05	< 0.05
Milk vs.		min	max	mean	min	max	mean		min	max	mean	min	max	mean
0	R^2	0.019	0.009	0.012	0.020	0.009	0.012	R^2	0.023	0.003	0.001	0.026	0.037	0.032
	P	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	P	> 0.05	> 0.05	> 0.05	> 0.05	< 0.05	< 0.05
1	R^2	0.049	0.034	0.048	0.050	0.038	0.050	R^2	0.065	0	0.011	0.067	0.025	0.042
	P	< 0.05	< 0.05	< 0.05	> 0.05	> 0.05	> 0.05	P	< 0.05	> 0.05	> 0.05	> 0.05	> 0.05	< 0.05
2	R^2	0.031	0.034	0.036	0.038	0.042	0.054	R^2	0.030	0.002	0	0.031	0.044	0.072
	P	< 0.05	< 0.05	< 0.05	> 0.05	> 0.05	> 0.05	P	< 0.05	> 0.05	> 0.05	> 0.05	< 0.05	< 0.05

Data set for regression analysis consisted of all 9 days during the progressive heat stress phase, for all cows, without averaging;
Highlighted cells indicate the best delay in response;
The P value below each R^2 value represents the significance of the linear or quadratic regressions.
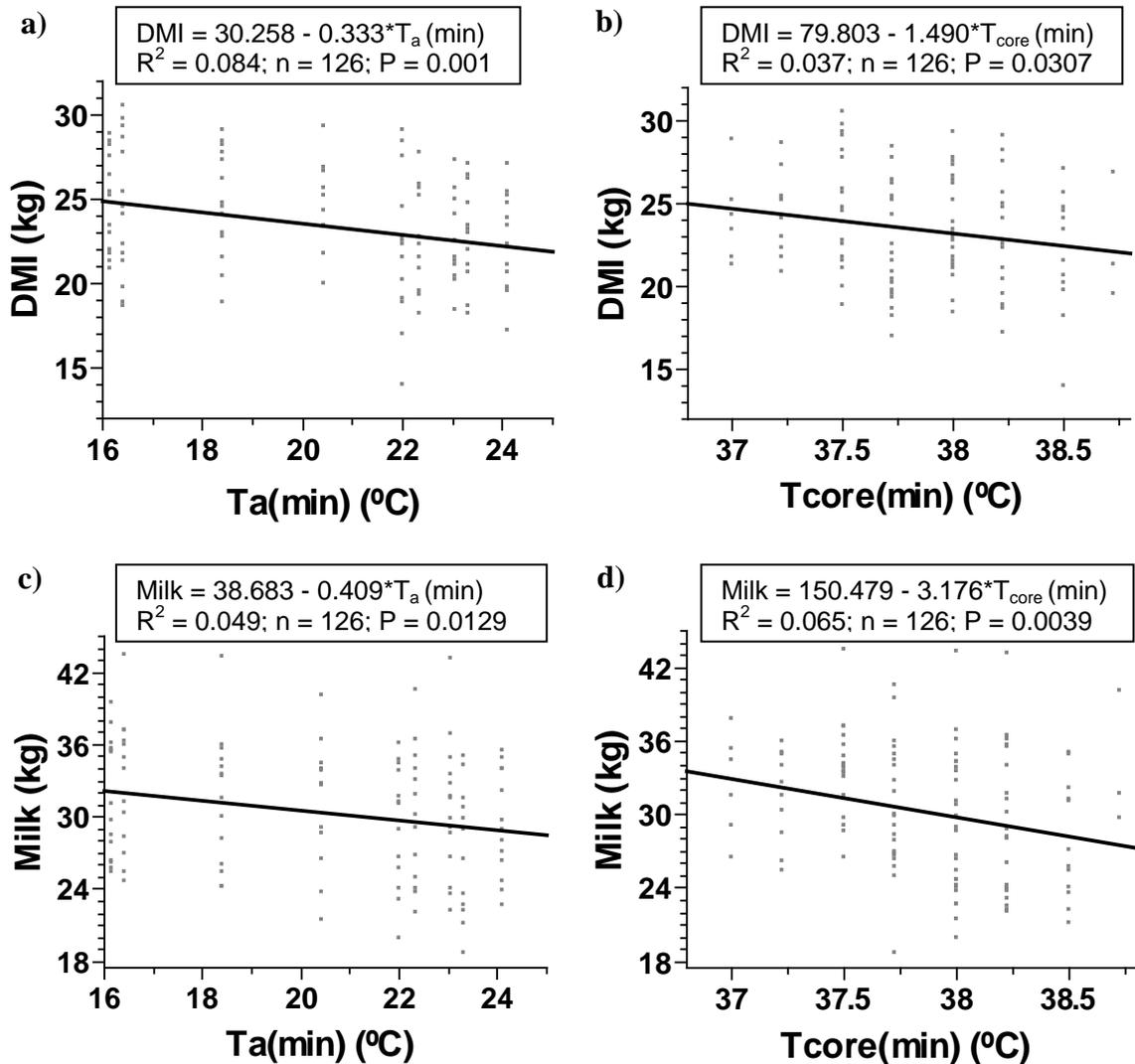
Figure 3.14. Linear regressions of DMI and milk production as functions of ambient temperature minimum (T_a(min)) and core body temperature minimum (T_{core}(min)), with the best delay in response for the relationship, during the progressive heat stress phase (d 32 to 40). They include: a) DMI vs. T_a(min) with no delay; b) DMI vs. T_{core}(min) with no delay; c) milk production vs. T_a(min) with 1 day delay; d) milk production vs. T_{core}(min) with 1 day delay. Each point represents daily values for all 9 days during the phase, for each individual animal. Information in the box refers to characteristics for linear regression through the points.

Table 3.11. Coefficients of determination (R^2) for the linear and quadratic regressions of milk production as a function of DMI, with delay in response ranging from 0 to 2 days, during the progressive heat stress phase (d 32 to 40)

Delay in response (d)		Linear	Quadratic
0	R^2	0.262	0.299
	P	*< 0.05*	*< 0.05*
1	R^2	0.326	0.339
	P	*< 0.05*	*> 0.05*
2	R^2	0.218	0.228
	P	*< 0.05*	*> 0.05*

Data set for regression analysis consisted of all 9 days during the progressive heat stress phase, for all cows, without averaging;

Highlighted cells indicate the best delay in response;

The P value below each R^2 value represents the significance of the linear or quadratic regressions.
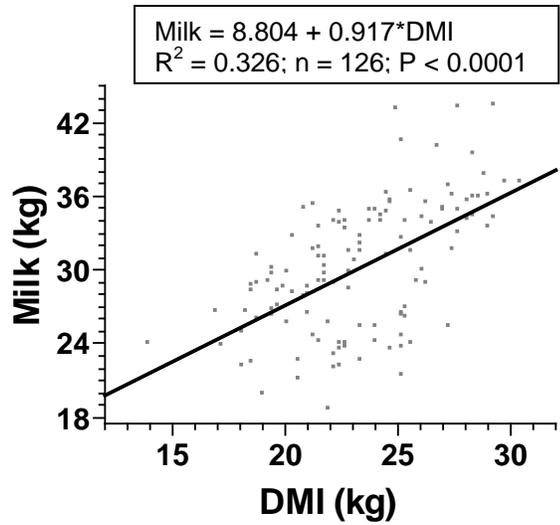
Figure 3.15. Linear regressions of milk production as functions of DMI, with 1 day delay, during the progressive heat stress phase (d 32 to 40). Each point represents daily values for all 9 days during the phase, for each individual animal. Information in the box refers to characteristics for linear regression through the points.

Table 3.12. Average DMI, milk production, respiration rate (RR), ear skin temperature (T_{ear}), rump skin temperature (T_{rump}) and core body temperature (T_{core}) for non-sensitive (NS) and sensitive (S) animals during the progressive heat stress phase (d 32 to 40)

Item	NS	S	SE_{pooled}	P value
DMI, kg/d	23.25	22.75	0.96	> 0.10
Milk, kg/d	30.75	29.37	1.35	> 0.10
RR, bpm	58.61	61.96	3.32	> 0.10
T_{ear}, ^{o}C	34.69	34.41	0.12	> 0.10
T_{rump}, ^{o}C	34.41	34.42	0.09	> 0.10
T_{core}, ^{o}C (whole day)	38.90	38.83	0.13	> 0.10
T_{core}, ^{o}C (1000 to 1500h)	38.82	38.83	0.14	> 0.10

Each sensitivity group consisted of 5 cows, identified through the R^2 value for the linear regression of T_{core} vs. T_a and DMI vs. T_{core}(min); cows with the lowest R^2 values for both relationships were assigned to NS and cows with the highest R^2 values for both relationships were assigned to S;

Values consisted of the average of the cows in each sensitivity group, for all 9 days during the progressive heat stress phase.
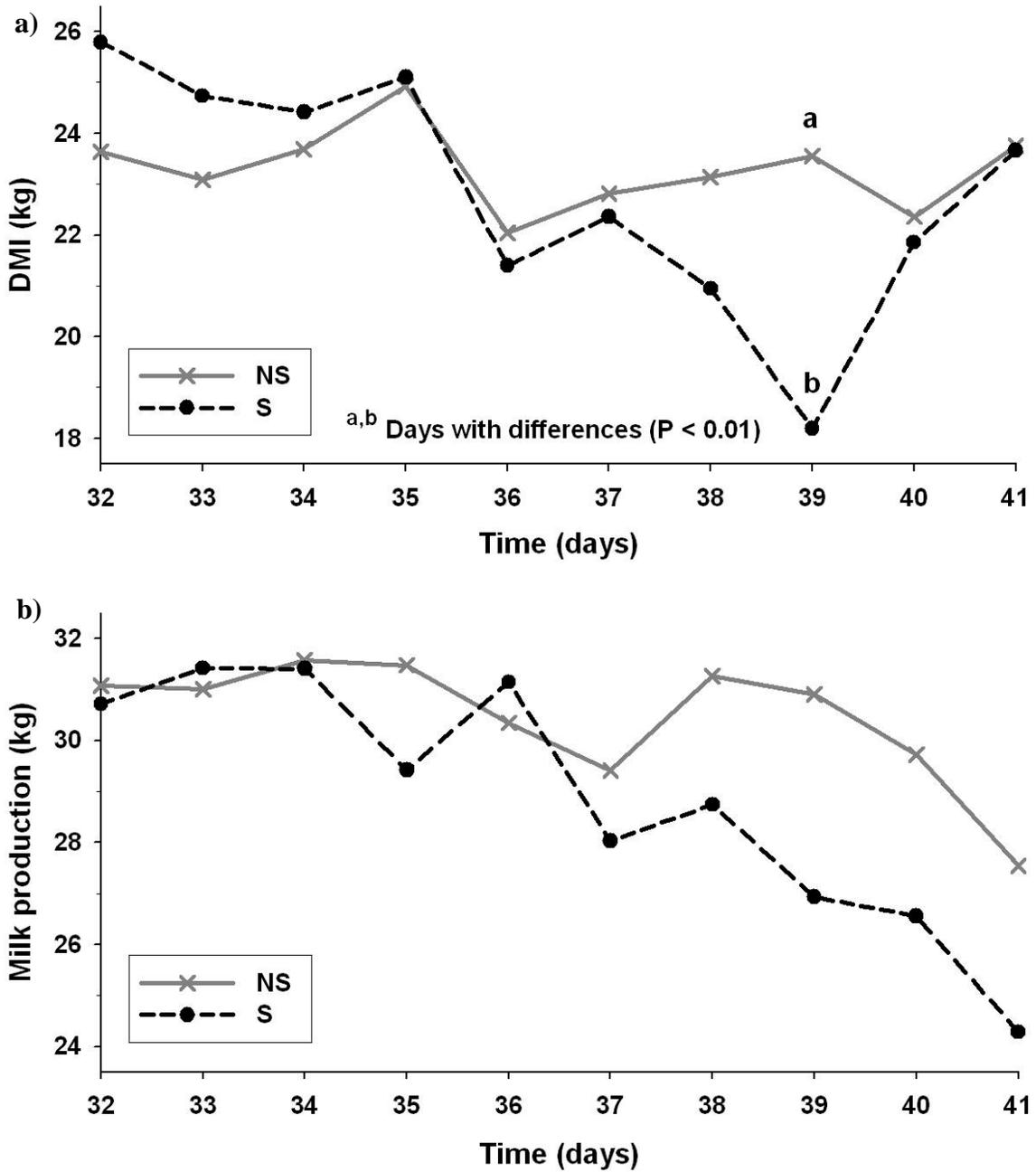
Figure 3.16. Average DMI (a) and milk production (b) for non-sensitive (NS) and sensitive (S) cows as a function of time (days), during the progressive heat stress phase (d 32 to 40). Sensitivity of cows was assessed with the linear regression of T_{core} vs. T_a and DMI vs. T_{core}(min); cows with the lowest R^2 values for both relationships were assigned to NS and cows with the highest R^2 values for both relationships were assigned to S.
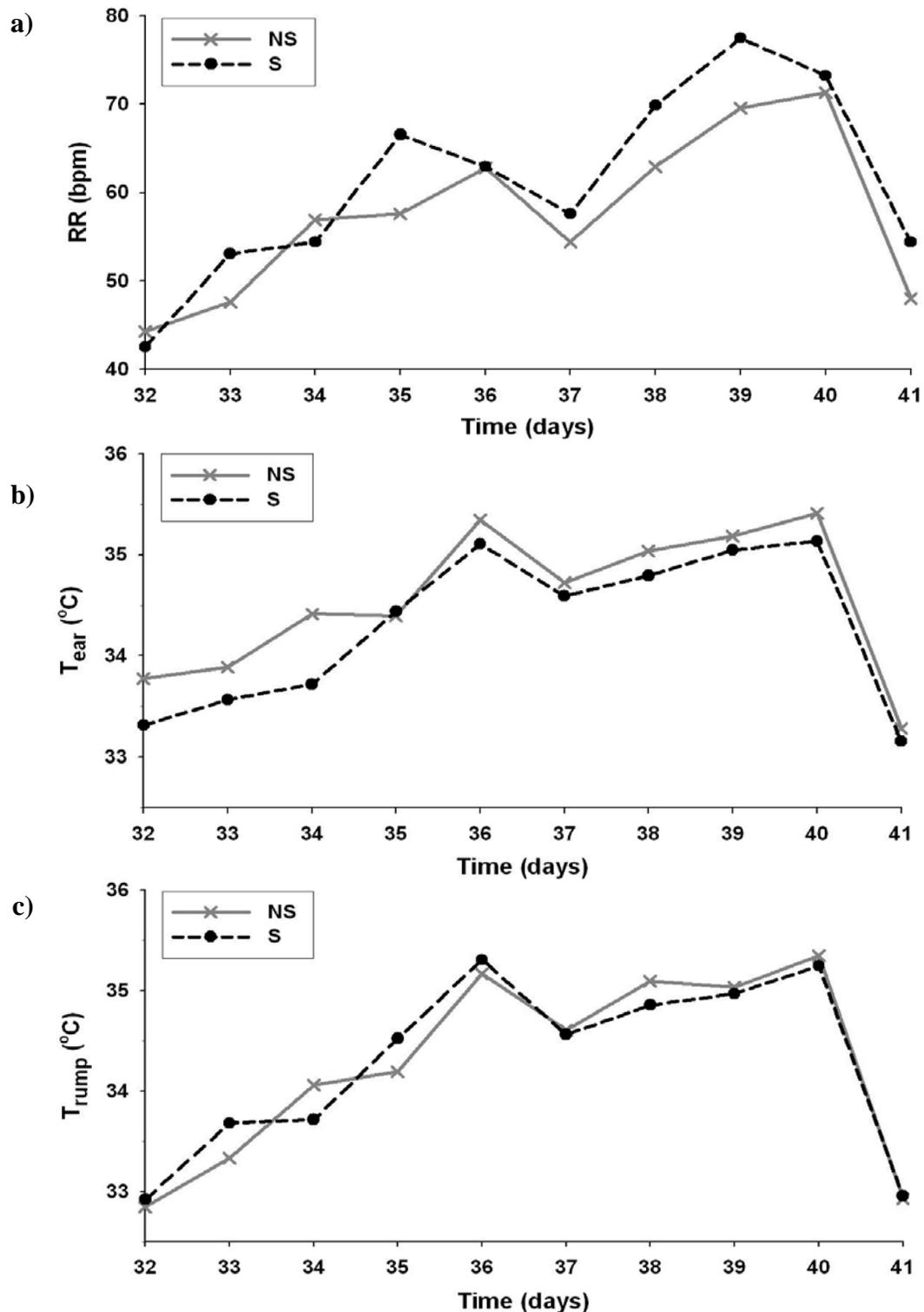
Figure 3.17. Thermal status of non-sensitive (NS) and sensitive (S) cows as a function of time (days), during the progressive heat stress phase (d 32 to 40). Figures a, b and c show respiration rate (RR), ear (T_{ear}) and rump skin temperatures (T_{rump}), respectively. Sensitivity of cows was assessed with the linear regression of T_{core} vs. T_a and DMI vs. T_{core}(min); cows with the lowest R^2 for both relationships were assigned to NS and cows with the highest R^2 were assigned to S.
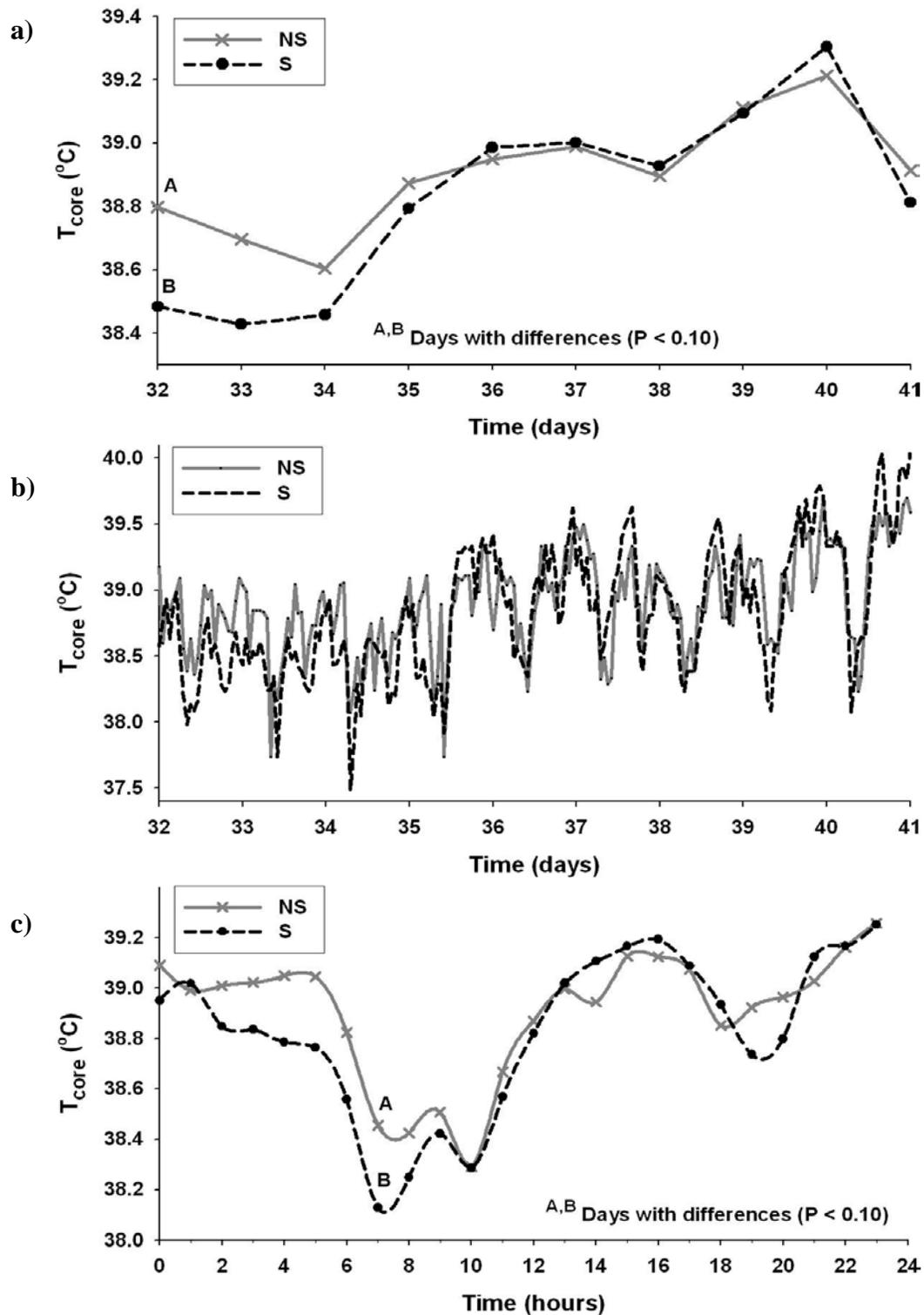
Figure 3.18. Core body temperature (T_{core}) of non-sensitive (NS) and sensitive (S) cows as a function of time, during the progressive heat stress phase (d 32 to 40). Figures a, b and c show T_{core} as daily averages, hourly measurements, and hourly averages, respectively. Sensitivity of cows was assessed with the linear regression of T_{core} vs. T_a and DMI vs. T_{core}(min); cows with the lowest R^2 for both relationships were assigned to NS and cows with the highest R^2 were assigned to S.

Table 3.13. Coefficients of determination (R^2) for linear and quadratic regressions of thermal status as functions of ambient temperature (T_a) and other thermal status, and of DMI and milk production as functions of minimum T_a and minimum core body temperature (T_{core}), for non-sensitive (NS), sensitive (S) and all animals, with the best delay for each relationship, during the progressive heat stress phase (d 32 to 40)

Variables (delay in response)		Linear			Quadratic		
		NS	S	All	NS	S	All
T_{core}[1] vs. T_a	R^2	0.104	0.303	0.221	0.104	0.304	0.222
(3 h delay)	P	*< 0.05*	*< 0.05*	*< 0.05*	*> 0.05*	*> 0.05*	*> 0.05*
T_{core}[2] vs. T_a	R^2	0.200	0.482	0.359	0.203	0.482	0.359
(2 h delay)	P	*< 0.05*	*< 0.05*	*< 0.05*	*> 0.05*	*> 0.05*	*> 0.05*
T_{core}[3] vs. T_a	R^2	0.222	0.416	0.327	0.244	0.427	0.338
(1 h delay)	P	*< 0.05*	*< 0.05*	*< 0.05*	*> 0.05*	*< 0.05*	*< 0.05*
RR vs. T_a	R^2	0.253	0.330	0.268	0.267	0.356	0.281
(0 h delay)	P	*< 0.05*	*< 0.05*	*< 0.05*	*> 0.05*	*> 0.05*	*< 0.05*
T_{ear} vs. T_a	R^2	0.378	0.341	0.352	0.403	0.374	0.380
(0 h delay)	P	*< 0.05*	*< 0.05*	*< 0.05*	*< 0.05*	*< 0.05*	*< 0.05*
T_{rump} vs. T_a	R^2	0.552	0.526	0.531	0.552	0.537	0.534
(0 h delay)	P	*< 0.05*	*< 0.05*	*< 0.05*	*> 0.05*	*> 0.05*	*> 0.05*
T_{core}[3] vs. RR	R^2	0.166	0.415	0.328	0.184	0.415	0.333
(1 h delay)	P	*< 0.05*	*< 0.05*	*< 0.05*	*> 0.05*	*> 0.05*	*> 0.05*
T_{core}[3] vs. T_{ear}	R^2	0.129	0.313	0.251	0.139	0.353	0.274
(1 h delay)	P	*< 0.05*	*< 0.05*	*< 0.05*	*> 0.05*	*< 0.05*	*< 0.05*
T_{core}[3] vs. T_{rump}	R^2	0.185	0.423	0.322	0.273	0.448	0.371
(1 h delay)	P	*< 0.05*	*< 0.05*	*< 0.05*	*< 0.05*	*< 0.05*	*< 0.05*
RR vs. T_{ear}	R^2	0.210	0.364	0.263	0.249	0.366	0.275
(0 h delay)	P	*< 0.05*	*< 0.05*	*< 0.05*	*< 0.05*	*> 0.05*	*< 0.05*
RR vs. T_{rump}	R^2	0.182	0.401	0.307	0.189	0.404	0.319
(0 h delay)	P	*< 0.05*	*< 0.05*	*< 0.05*	*> 0.05*	*> 0.05*	*< 0.05*
T_{rump} vs. T_{ear}	R^2	0.580	0.573	0.580	0.608	0.581	0.601
(0 h delay)	P	*< 0.05*	*< 0.05*	*< 0.05*	*< 0.05*	*> 0.05*	*< 0.05*
DMI vs. T_a(min)	R^2	0.021	0.210	0.084	0.068	0.213	0.091
(0 d delay)	P	*> 0.05*	*< 0.05*	*< 0.05*	*> 0.05*	*> 0.05*	*> 0.05*
DMI vs. T_{core}(min)	R^2	0.007	0.132	0.037	0.022	0.132	0.038
(0 d delay)	P	*> 0.05*	*< 0.05*	*< 0.05*	*> 0.05*	*> 0.05*	*> 0.05*
Milk vs. T_a(min)	R^2	0.015	0.130	0.049	0.016	0.143	0.050
(1 d delay)	P	*> 0.05*	*< 0.05*	*< 0.05*	*> 0.05*	*> 0.05*	*> 0.05*
Milk vs. T_{core}(min)	R^2	0.130	0.098	0.065	0.140	0.098	0.067
(1 d delay)	P	*< 0.05*	*< 0.05*	*< 0.05*	*> 0.05*	*> 0.05*	*> 0.05*

[1] T_{core} values for whole day; [2] T_{core} values from 1000 to 1500 h; [3] T_{core} values at 0700, 1600 and 1900 h;
Each sensitivity group consisted of 5 cows, identified through the R^2 value for the linear regression of T_{core} vs. T_a and DMI vs. T_{core}(min); cows with the lowest R^2 values for both relationships were assigned to NS and cows with the highest R^2 values for both relationships were assigned to S;
Data set for regression analysis consisted of hourly or daily values of all 9 days during the progressive heat stress phase, for all cows in each sensitivity group, without averaging;
The P value below each R^2 value represents the significance of the linear or quadratic regressions.
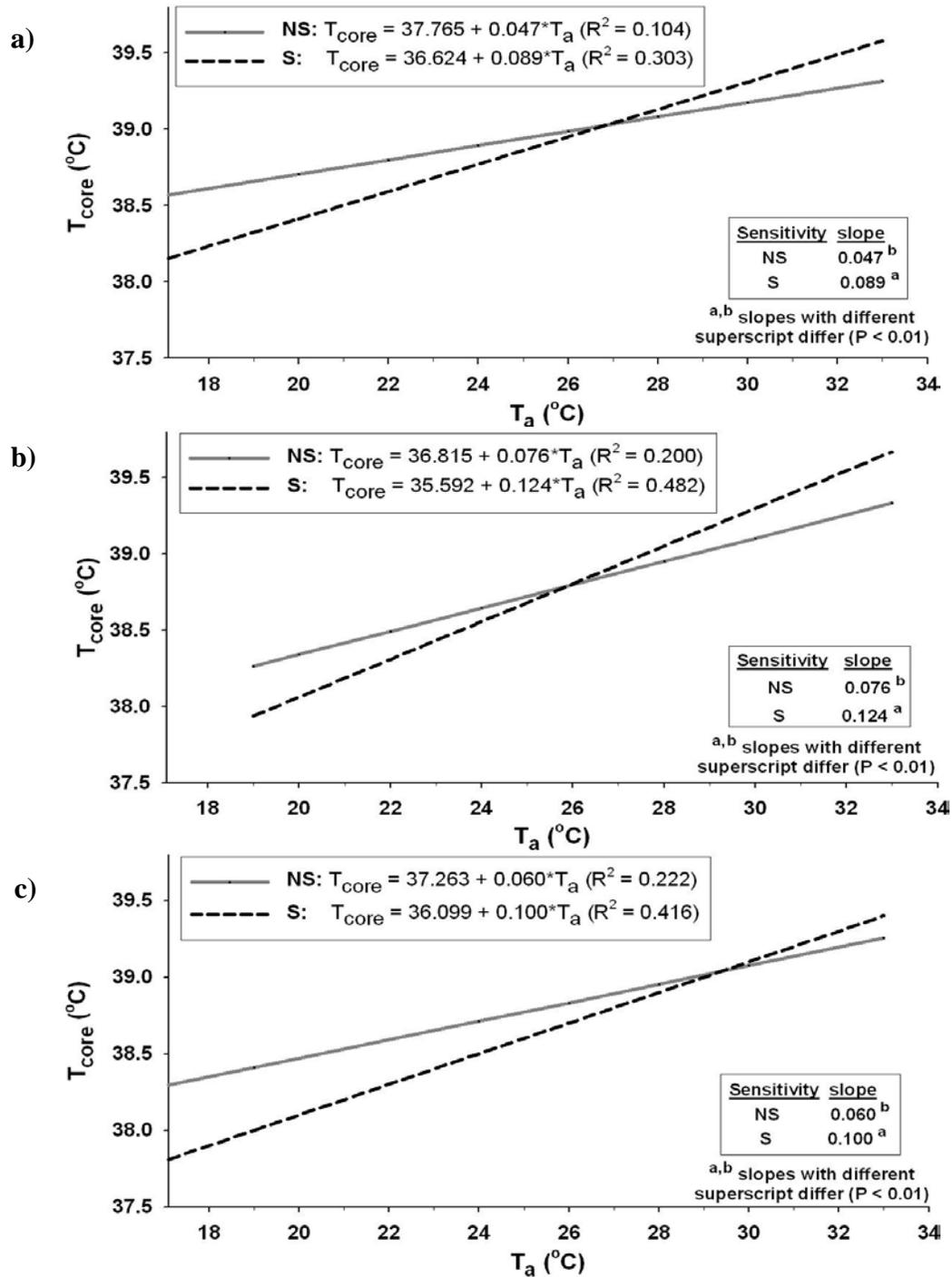
Figure 3.19. Linear regressions of core body temperature (T_{core}) as a function of ambient temperature (T_a) for non-sensitive (NS) and sensitive (S) cows, with the best delay in response for the relationship, during the progressive heat stress phase (d 32 to 40). They include: a) T_{core} for the whole day with 3 hours delay; b) T_{core} from 1000 to 1500 h with 2 hours delay; c) T_{core} at 0700, 1600 and 1900 h with 1 hour delay. Sensitivity of cows was assessed with the linear regression of T_{core} vs. T_a and DMI vs. T_{core}(min); cows with the lowest R^2 for both relationships were assigned to NS and cows with the highest R^2 were assigned to S. Data set for regression analysis consisted of hourly values of all 9 days during the progressive heat stress phase, for all cows in each sensitivity group, without averaging.
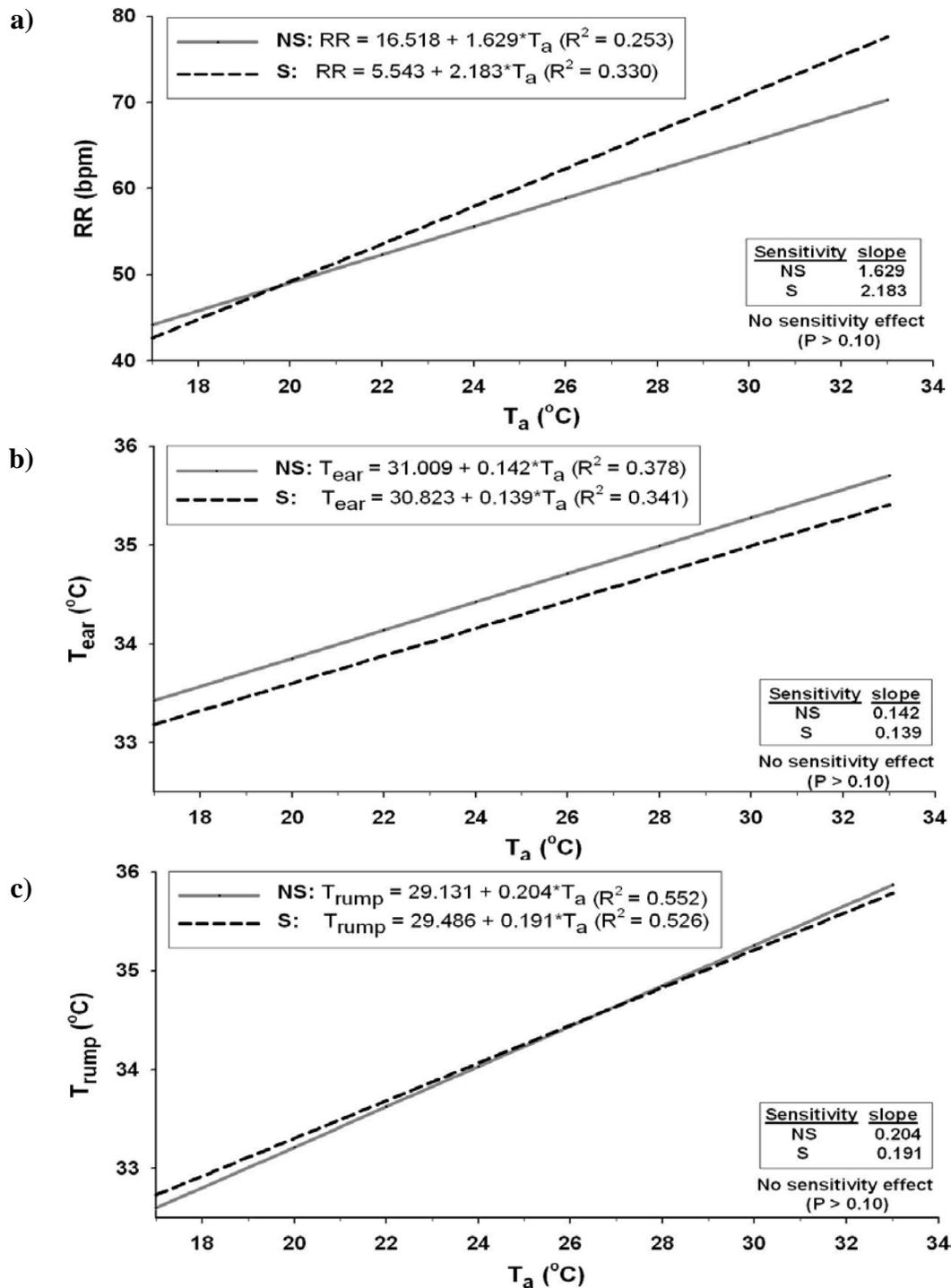
Figure 3.20. Linear regressions of thermal status of non-sensitive (NS) and sensitive (S) cows as a function of ambient temperature (T_a), with no delay in response, during the progressive heat stress phase (d 32 to 40). Figures a, b and c show respiration rate (RR), ear (T_{ear}) and rump skin temperatures (T_{rump}), respectively. Sensitivity of cows was assessed with the linear regression of T_{core} vs. T_a and DMI vs. T_{core}(min); cows with the lowest R^2 for both relationships were assigned to NS and cows with the highest R^2 were assigned to S. Data set for regression analysis consisted of hourly values of all 9 days during the progressive heat stress phase, for all cows in each sensitivity group, without averaging.

a)

NS: T_{core} = 37.886 + 0.016*RR (R^2 = 0.166)
S: T_{core} = 37.123 + 0.026*RR (R^2 = 0.415)

Sensitivity	slope
NS	0.016 [b]
S	0.026 [a]

[a,b] slopes with different superscript differ ($P < 0.05$)

b)

NS: T_{core} = 31.438 + 0.213*T_{ear} (R^2 = 0.129)
S: T_{core} = 26.474 + 0.356*T_{ear} (R^2 = 0.313)

Sensitivity	slope
NS	0.213 [b]
S	0.356 [a]

[a,b] slopes with different superscript differ ($P < 0.05$)

c)

NS: T_{core} = 31.757 + 0.205*T_{rump} (R^2 = 0.185)
S: T_{core} = 28.843 + 0.374*T_{rump} (R^2 = 0.423)

Sensitivity	slope
NS	0.205 [b]
S	0.374 [a]

[a,b] slopes with different superscript differ ($P < 0.05$)

Figure 3.21. Linear regressions of core body temperature (T_{core}) of non-sensitive (NS) and sensitive (S) cows as a function of other thermal status, with 1 hour delay, during the progressive heat stress phase (d 32 to 40). Figures a, b and c show T_{core} as a function of respiration rate (RR), ear (T_{ear}) and rump skin temperatures (T_{rump}), respectively. Sensitivity of cows was assessed with the linear regression of T_{core} vs. T_a and DMI vs. T_{core}(min); cows with the lowest R^2 for both relationships were assigned to NS and cows with the highest R^2 were assigned to S. Data set for regression analysis consisted of hourly values of all 9 days during the progressive heat stress phase, for all cows in each sensitivity group, without averaging.

168
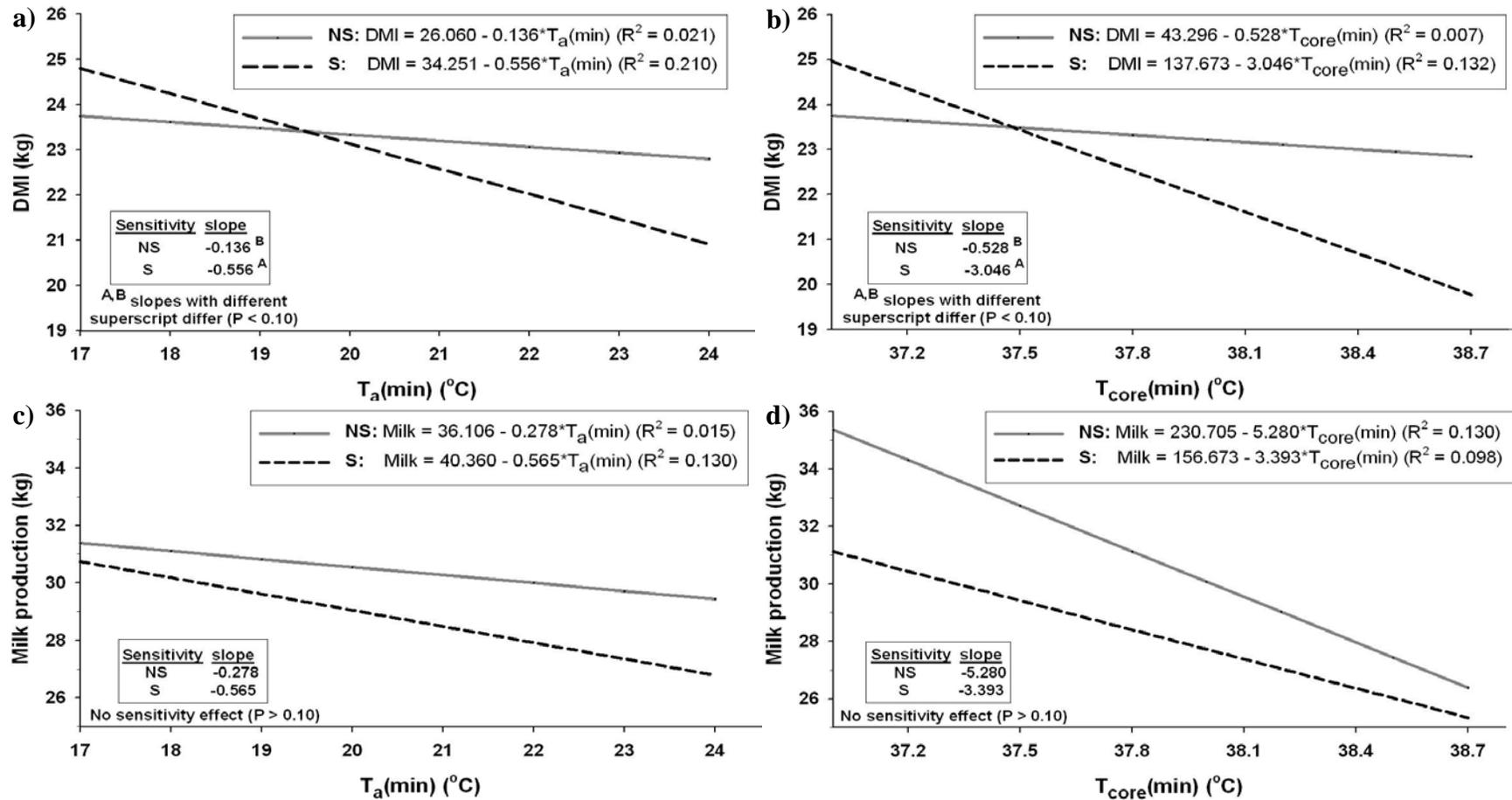
Figure 3.22. Linear regressions of DMI and milk production of non-sensitive (NS) and sensitive (S) cows as functions of minimum ambient temperature (T_a(min)) and minimum core body temperature (T_{core}(min)), with the best delay in response for the relationship, during the progressive heat stress phase (d 32 to 40). They include: a) DMI vs. T_a(min) with no delay; b) DMI vs. T_{core}(min) with no delay; c) milk production vs. T_a(min) with 1 day delay; d) milk production vs. T_{core}(min) with 1 day delay. Sensitivity of cows was assessed with the linear regression of T_{core} vs. T_a and DMI vs. T_{core}(min); cows with the lowest R^2 for both relationships were assigned to NS and cows with the highest R^2 were assigned to S. Data set for regression analysis consisted of daily values of all 9 days during the progressive heat stress phase, for all cows in each sensitivity group, without averaging.

LITERATURE CITED

Allen, V. G., K. R. Pond, K. E. Saker, J. P. Fontenot, C. P. Bagley, R. L. Ivy, R. R. Evans, C. P. Brown, M. F. Miller, J. L. Montgomery, T. M. Dettle, and D. B. Wester. 2001a. Tasco-forage: Iii. Influence of a seaweed extract on performance, monocyte immune cell response, and carcass characteristics in feedlot-finished steers. J. Anim Sci. 79: 1032-1040.

Allen, V. G., K. R. Pond, K. E. Saker, J. P. Fontenot, C. P. Bagley, R. L. Ivy, R. R. Evans, R. E. Schmidt, J. H. Fike, X. Zhang, J. Y. Ayad, C. P. Brown, M. F. Miller, J. L. Montgomery, J. Mahan, D. B. Wester, and C. Melton. 2001b. Tasco: Influence of a brown seaweed on antioxidants in forages and livestock--a review. J. Anim Sci. 79: E21-31.

Anderson, M. J., J. R. Blanton, Jr., J. Gleghorn, S. W. Kim, and J. W. Johnson. 2006. Ascophyllum nodosum supplementation strategies that improve overall carcass merit of implanted english crossbred cattle. Asian-Australas. J. Anim Sci. 19: 1514-1518.

AOAC. 1995. Official Methods of Analysis. 16th ed. Association of Official Analytical Chemists, Washington, DC.

Araki, C. T., R. M. Nakamura, L. W. G. Kam, and N. Clarke. 1984. Effect of lactation on diurnal temperature patterns of dairy cattle in hot environments. J. Dairy Sci. 67: 1752-1760.

Archer, G. 2005. Reducing stress in sheep by feeding the seaweed *Ascophyllum nodosum.* Ph.D. dissertation. Texas A&M University, College Station.

Archer, G. S., T. H. Friend, D. Caldwell, K. Ameiss, and P. D. Krawczel. 2007. Effect of the seaweed ascophyllum nodosum on lambs during forced walking and transport. J. Anim Sci. 85: 225-232.

Archer, G. S., T. H. Friend, D. Caldwell, K. Ameiss, P. D. Krawczel, C. Iacono, H. Keen, and T. Martin. 2008. Impacts of feeding several components of the seaweed ascophyllum nodosum on transported lambs. Anim. Feed Sci. Technol. 140: 258-271.

Armstrong, D. V. 1994. Heat stress interaction with shade and cooling. J. Dairy Sci. 77: 2044-2050.

Bach, S. J., Y. Wang, and T. A. McAllister. 2008. Effect of feeding sun-dried seaweed (ascophyllum nodosum) on fecal shedding of escherichia coli o157:H7 by feedlot cattle and on growth performance of lambs. Anim. Feed Sci. Technol. 142: 17-32.

Behrends, L. L., J. R. Blanton, Jr., M. F. Miller, K. R. Pond, and V. G. Allen. 2000. Tasco supplementation in feedlot cattle: Effects on pathogen loads. J. Anim. Sci. 78(Suppl. 1):106 (Abstr.)

Berman, A. 1971. Thermoregulation in intensively lactating cows in near-natural conditions. The Journal of Physiology 215: 477-489.

Berman, A. 2005. Estimates of heat stress relief needs for holstein dairy cows. J. Anim Sci. 83: 1377-1384.

Berman, A., Y. Folman, M. Kaim, M. Mamen, Z. Herz, D. Wolfenson, A. Arieli, and Y. Graber. 1985. Upper critical temperatures and forced ventilation effects for high-yielding dairy cows in a subtropical climate. J. Dairy Sci. 68: 1488-1495.

Bernabucci, U., P. Bani, B. Ronchi, N. Lacetera, and A. Nardone. 1999. Influence of short- and long-term exposure to a hot environment on rumen passage rate and diet digestibility by friesian heifers. J. Dairy Sci. 82: 967-973.

Bernabucci, U., N. Lacetera, P. P. Danieli, P. Bani, A. Nardone, and B. Ronchi. 2009. Influence of different periods of exposure to hot environment on rumen function and diet digestibility in sheep. Int. J. Biometeorol. 53: 387-395.

Boehmer, B. H., C. L. Bailey, E. C. Wright, and R. P. Wettemann. 2009. Effects of temperature of consumed water on rumen temperature of beef cows. Available on line at http://www.ansi.okstate.edu/research/research-reports-1/2009/2009%20Boehmer%20Research%20Report.pdf Accessed March 12, 2010.

Bohmanova, J., I. Misztal, and J. B. Cole. 2007. Temperature-humidity indices as indicators of milk production losses due to heat stress. J. Dairy Sci. 90: 1947-1956.

Braden, K. W., J. R. Blanton, Jr., V. G. Allen, K. R. Pond, and M. F. Miller. 2004. Ascophyllum nodosum supplementation: A preharvest intervention for reducing escherichia coli o157:H7 and salmonella spp. In feedlot steers. J. Food Prot. 67: 1824-1828.

Braden, K. W., J. R. Blanton, Jr., J. L. Montgomery, E. van Santen, V. G. Allen, and M. F. Miller. 2007. Tasco supplementation: Effects on carcass characteristics, sensory attributes, and retail display shelf-life. J. Anim Sci. 85: 754-768.

Brown-Brandl, T. M., R. A. Eigenberg, G. L. Hahn, J. A. Nienaber, T. L. Mader, D. E. Spiers, and A. M. Parkhurst. 2005a. Analyses of thermoregulatory responses of feeder cattle exposed to simulated heat waves. Int. J. Biometeorol. 49: 285-296.

Brown-Brandl, T. M., R. A. Eigenberg, J. A. Nienaber, and G. L. Hahn. 2005b. Dynamic response indicators of heat stress in shaded and non-shaded feedlot cattle, part 1: Analyses of indicators. Biosys. Eng. 90: 451-462.

Brown-Brandl, T. M., J. A. Nienaber, R. A. Eigenberg, G. L. Hahn, and H. Freetly. 2003. Thermoregulatory responses of feeder cattle. J. Therm. Biol. 28: 149-157.

Burtin, P. 2003. Nutritional value of seaweeds. Electronic Journal of Environmental, Agricultural and Food Chemistry 2: paper 17.

Collier, R. J., G. E. Dahl, and M. J. VanBaale. 2006. Major advances associated with environmental effects on dairy cattle. J. Dairy Sci. 89: 1244-1253.

Collier, R. J., R. M. Eley, A. K. Sharma, R. M. Pereira, and D. E. Buffington. 1981. Shade management in subtropical environment for milk yield and composition in holstein and jersey cows. J. Dairy Sci. 64: 844-849.

Collier, R. J., and R. B. Zimbelman. 2007. Heat stress effects on cattle: What we know and what we don't know. Pages 76–83 in 22nd Annu. Southwest Nutr. Manage. Conf., Tempe, AZ. Univ. Arizona, Tempe.

Cvetkovic, B., M. J. Brouk, and J. E. Shirley. 2005. Response of heat stressed lactating dairy cattle fed dried seaweed meal. J. Dairy Sci. 88:1920 (Abstr.)

DHI Info sheet. Persistency of milk production. Western Canadian Dairy Herd Improvement Services. Available on line at http://www.agromedia.ca/ADM_Articles/content/DHI_persist.pdf. Accessed May 13, 2010.

Dikmen, S., and P. J. Hansen. 2009. Is the temperature-humidity index the best indicator of heat stress in lactating dairy cows in a subtropical environment? J. Dairy Sci. 92: 109-116.

Drackley, J. K., T. M. Cicela, and D. W. LaCount. 2003. Responses of primiparous and multiparous holstein cows to additional energy from fat or concentrate during summer. J. Dairy Sci. 86: 1306-1314.

Eichen, P. A., M. J. Leonard, M. A. Kozma, B. M. Kronk, L. E. McVicker, D. E. Spiers, and D. P. Colling. 2003. Is iodide responsible for the heat-relief effects of Ascophyllum nodosum? J. Anim. Sci. 81(Suppl. 1):155 (Abstr.)

Eichen, P. A., D. E. Spiers, G. E. Rottinghaus, and D. P. Colling. 2001. Utilization of a small animal model of fescue toxicosis to evaluate the potential benefit of *Ascophyllum nodosum*. J. Anim. Sci. 79(Suppl. 1):16 (Abstr.)

Fike, J. H., V. G. Allen, R. E. Schmidt, X. Zhang, J. P. Fontenot, C. P. Bagley, R. L. Ivy, R. R. Evans, R. W. Coelho, and D. B. Wester. 2001. Tasco-forage: I. Influence of a seaweed extract on antioxidant activity in tall fescue and in ruminants. J. Anim Sci. 79: 1011-1021.

Finch, V. A. 1986. Body temperature in beef cattle: Its control and relevance to production in the tropics. J. Anim Sci. 62: 531-542.

Fox, D. G., and T. P. Tylutki. 1998. Accounting for the effects of environment on the nutrient requirements of dairy cattle. J. Dairy Sci. 81: 3085-3095.

Gaughan, J. B., S. M. Holt, G. L. Hahn, T. L. Mader, and R. Eigenberg. 2000. Respiration rate - is it a good measure of heat stress in cattle? Asian-Australas. J. Anim Sci. 13: 329-332.

Gaughan, J. B., T. L. Mader, S. M. Holt, and A. Lisle. 2007. A new heat load index for feedlot cattle. J. Anim Sci.: jas.2007-0305.

Hahn, G. L. 1999. Dynamic responses of cattle to thermal heat loads. J. Anim Sci. 77: 10-20.

Hahn, G. L., R. A. Eigenberg, J. A. Nienaber, and E. T. Littledike. 1990. Measuring physiological responses of animals to environmental stressors using a microcomputer-based portable datalogger. J. Anim Sci. 68: 2658-2665.

Holter, J. B., J. W. West, M. L. McGilliard, and A. N. Pell. 1996. Predicting ad libitum dry matter intake and yields of jersey cows. J. Dairy Sci. 79: 912-921.

Huber, J. T., G. Higginbotham, R. A. Gomez-Alarcon, R. B. Taylor, K. H. Chen, S. C. Chan, and Z. Wu. 1994. Heat stress interactions with protein supplemental fat, and fungal cultures. J. Dairy Sci. 77: 2080-2090.

Igono, M. O., and H. D. Johnson. 1990. Physiologic stress index of lactating dairy cows based on diurnal pattern of rectal temperature. J. Interdiscip. Cycle Res. 21: 303-320.

Jensen, A. 1969. Tocopherol content of seaweed and seaweed meal. 2. Individual, diurnal and seasonal variations in some fucaceae. J. Sci. Food Agric. 20: 454-458.

Johnson, H. D. 1965. Environmental temperature and lactation (with special reference to cattle). Int. J. Biometeorol. 9: 103-116.

Jones, G. M., and C. C. Stallings. 1999. Reducing heat stress for dairy cattle. Dep. Dairy Sci., Virginia Tech. Univ. Publication Number 404-200. Available on line at http://hydrofun.net/pdf/dairy_misting.pdf. Accessed March 26, 2009.

Kadzere, C. T., M. R. Murphy, N. Silanikove, and E. Maltz. 2002. Heat stress in lactating dairy cows: A review. Livest. Prod. Sci. 77: 59-91.

Kelley, K. W. 1980. Stress and immune function: A bibliographic review. Annales de Recherches Veterinaires 11: 445-478.

Kelley, R. O., F. A. Martz, and H. D. Johnson. 1967. Effect of environmental temperature on ruminal volatile fatty acid levels with controlled feed intake. J. Dairy Sci. 50: 531-533.

Kellogg, D. W., J. A. Pennington, Z. B. Johnson, K. S. Anschutz, D. P. Colling, and A. B. Johnson. 2006. Effects of feeding Tasco Ascophyllum nodosum to large and small dairy cows during summer months in central Arkansas. J. Anim. Sci. 84(Suppl. 1):72 (Abstr.)

Kendall, P. E., P. P. Nielsen, J. R. Webster, G. A. Verkerk, R. P. Littlejohn, and L. R. Matthews. 2006. The effects of providing shade to lactating dairy cows in a temperate climate. Livest. Sci. 103: 148-157.

Knights, B. A. 1970. Sterols in ascophyllum nodosum. Phytochemistry 9: 903-905.

Linvill, D. E., and F. E. Pardue. 1992. Heat stress and milk production in the south carolina coastal plains. J. Dairy Sci. 75: 2598-2604.

Littell, R. C., P. R. Henry, and C. B. Ammerman. 1998. Statistical analysis of repeated measures data using sas procedures. J. Anim Sci. 76: 1216-1231.

Mader, T., J. Gaughan, L. Johnson, and G. Hahn. 2009. Tympanic temperature in confined beef cattle exposed to excessive heat load. Int. J. Biometeorol.

Mader, T. L., M. S. Davis, and T. Brown-Brandl. 2006. Environmental factors influencing heat stress in feedlot cattle. J. Anim Sci. 84: 712-719.

Mader, T. L., S. M. Holt, G. L. Hahn, M. S. Davis, and D. E. Spiers. 2002. Feeding strategies for managing heat load in feedlot cattle. J. Anim Sci. 80: 2373-2382.

Maust, L. E., R. E. Macdowell, and N. W. Hooven. 1972. Effect of summer weather on performance of holstein cows in three stages of lactation. J. Dairy Sci. 55: 1133-1139.

Moallem, U., H. Lehrer, L. Livshitz, M. Zachut, and S. Yakoby. 2009. The effects of live yeast supplementation to dairy cows during the hot season on production, feed efficiency, and digestibility. J. Dairy Sci. 92: 343-351.

Montgomery, J. L., V. G. Allen, K. R. Pond, M. F. Miller, D. B. Wester, C. P. Brown, R. Evans, C. P. Bagley, R. L. Ivy, and J. P. Fontenot. 2001. Tasco-forage: Iv. Influence of a seaweed extract applied to tall fescue pastures on sensory characteristics, shelf-life, and vitamin e status in feedlot-finished steers. J. Anim Sci. 79: 884-894.

Moore, C. E., J. K. Kay, R. J. Collier, M. J. VanBaale, and L. H. Baumgard. 2005. Effect of supplemental conjugated linoleic acids on heat-stressed brown swiss and holstein cows. J. Dairy Sci. 88: 1732-1740.

Muller, C. J. C., J. A. Botha, W. A. Coetzer, and W. A. Smith. 1994a. Effect of shade on various parameters of friesian cows in a mediterranean climate in south africa. 2. Physiological responses. S. Afr. J. Anim. Sci. 24: 56-60.

Muller, C. J. C., J. A. Botha, and W. A. Smith. 1994b. Effect of shade on various parameters of friesian cows in a mediterranean climate in south africa. 1. Feed and water intake, milk production and milk composition. S. Afr. J. Anim. Sci. 24: 49-55.

Muller, C. J. C., J. A. Botha, and W. A. Smith. 1994c. Effect of shade on various parameters of friesian cows in a mediterranean climate in south africa. 3. Behaviour. S. Afr. J. Anim. Sci. 24: 61-66.

Nardone, A., B. Ronchi, N. Lacetera, M. S. Ranieri, and U. Bernabucci. 2010. Effects of climate changes on animal production and sustainability of livestock systems. Livest. Sci. 130: 57-69.

National Mastitis Council. 2002. Recommended milking procedures. National Mastitis Council, Madison, WI.

Olde Riekerink, R. G. M., H. W. Barkema, and H. Stryhn. 2007. The effect of season on somatic cell count and the incidence of clinical mastitis. J. Dairy Sci. 90: 1704-1715.

Oliver, J. C., H. M. Hellman, S. E. Bishop, C. L. Pelissier, and L. F. Bennett. 1979. Heat stress survey. Calif Agric 33: 6-8.

Orth, R. 1992. Sample Day and Lactation Report. DHIA 200 Fact Sheet A-2. Mid-States DRPC, Ames, IA.

Ravagnolo, O., I. Misztal, and G. Hoogenboom. 2000. Genetic component of heat stress in dairy cattle, development of heat index function. J. Dairy Sci. 83: 2120-2125.

Rhoads, M. L., J. W. Kim, R. J. Collier, B. A. Crooker, Y. R. Boisclair, L. H. Baumgard, and R. P. Rhoads. 2010. Effects of heat stress and nutrition on lactating holstein cows: Ii. Aspects of hepatic growth hormone responsiveness. J. Dairy Sci. 93: 170-179.

Rhoads, M. L., R. P. Rhoads, M. J. VanBaale, R. J. Collier, S. R. Sanders, W. J. Weber, B. A. Crooker, and L. H. Baumgard. 2009. Effects of heat stress and plane of nutrition on lactating holstein cows: I. Production, metabolism, and aspects of circulating somatotropin. J. Dairy Sci. 92: 1986-1997.

Roenfeldt, S. 1998. You can't afford to ignore heat stress. Dairy Manag. 35: 6-12.

Saker, K. E., V. G. Allen, J. P. Fontenot, C. P. Bagley, R. L. Ivy, R. R. Evans, and D. B. Wester. 2001. Tasco-forage: Ii. Monocyte immune cell response and performance of beef steers grazing tall fescue treated with a seaweed extract. J. Anim Sci. 79: 1022-1031.

Saker, K. E., J. H. Fike, H. Veit, and D. L. Ward. 2004. Brown seaweed- (Tasco^{TM}) treated conserved forage enhances antioxidant status and immune function in heat-stressed wether lambs. J. Anim. Physiol. Anim. Nutr. 88: 122-130.

Schingoethe, D. J., K. N. Linke, K. F. Kalscheur, A. R. Hippen, D. R. Rennich, and I. Yoon. 2004. Feed efficiency of mid-lactation dairy cows fed yeast culture during summer. J. Dairy Sci. 87: 4178-4181.

Schneider, P. L., D. K. Beede, and C. J. Wilcox. 1988. Nycterohemeral patterns of acid-base status, mineral concentrations and digestive function of lactating cows in natural or chamber heat stress environments. J. Anim Sci. 66: 112-125.

Sharp, G. 2005. *Ascophyllum nodosum* and its harvesting in Eastern Canada. Available on line at http://www.fao.org/docrep/X5819E/x5819e04.htm. Accessed Feb. 19, 2010.

Shwartz, G., M. L. Rhoads, M. J. VanBaale, R. P. Rhoads, and L. H. Baumgard. 2009. Effects of a supplemental yeast culture on heat-stressed lactating holstein cows. J. Dairy Sci. 92: 935-942.

Spiers, D. E., P. A. Eichen, M. J. Leonard, L. E. Wax, G. E. Rottinghaus, J. E. Williams, and D. P. Colling. 2004a. Benefit of dietary seaweed (ascophyllum nodosum) extract in reducing heat strain and fescue toxicosis: A comparative evaluation. J. Therm. Biol. 29: 753-757.

Spiers, D. E., L. E. McVicker, J. E. Williams, P. A. Eichen, L. Thompson, G. Rottinghaus, and D. P. Colling. 2003. Monitoring fescue toxicosis in a pasture environment and evaluating the effect of prior treatment with *Ascophyllum nodosum*. J. Anim. Sci. 81(Suppl. 1):156 (Abstr.)

Spiers, D. E., J. N. Spain, J. D. Sampson, and R. P. Rhoads. 2004b. Use of physiological parameters to predict milk yield and feed intake in heat-stressed dairy cows. J. Therm. Biol. 29: 759-764.

Staples, C. R. 2007. Nutrient and feeding strategies to enable cows to cope with heat stress conditions. Pages 93–108 in 22nd Annu. Southwest Nutr. Manage. Conf., Tempe, AZ. Univ. Arizona, Tempe.

St-Pierre, N. R., B. Cobanov, and G. Schnitkey. 2003. Economic losses from heat stress by us livestock industries. J. Dairy Sci. 86: E52-77.

Tapkı, I., and A. Şahin. 2006. Comparison of the thermoregulatory behaviours of low and high producing dairy cows in a hot environment. Appl. Anim. Behav. Sci. 99: 1-11.

Thom, E. C. 1959. The discomfort index. Weatherwise. 12: 57-59.

Thompson, L. N. 2004. The effects of *Ascophyllum nodosum* for alleviation of heat stress in cattle. M.S. thesis. University of Missouri.

Turner, J. L., S. S. Dritz, J. J. Higgins, and J. E. Minton. 2002. Effects of ascophyllum nodosum extract on growth performance and immune function of young pigs challenged with salmonella typhimurium. J. Anim Sci. 80: 1947-1953.

Vacca, D. D., and R. A. Walsh. 1954. The antibacterial activity of an extract obtained from *ascophyllum nodosum*. J. Am. Pharm. Assoc. 43: 24-26.

Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74: 3583-3597.

West, J. W. 2003. Effects of heat-stress on production in dairy cattle. J. Dairy Sci. 86: 2131-2144.

West, J. W., B. G. Mullinix, and J. K. Bernard. 2003. Effects of hot, humid weather on milk temperature, dry matter intake, and milk yield of lactating dairy cows. J. Dairy Sci. 86: 232-242.

West, J. W., B. G. Mullinix, and T. G. Sandifer. 1991. Changing dietary electrolyte balance for dairy cows in cool and hot environments. J. Dairy Sci. 74: 1662-1674.

Wilks, D. L., C. E. Coppock, J. K. Lanham, K. N. Brooks, C. C. Baker, W. L. Bryson, R. G. Elmore, and R. A. Stermer. 1990. Responses of lactating holstein cows to chilled drinking water in high ambient temperatures. J. Dairy Sci. 73: 1091-1099.

Williams, J. E., D. E. Spiers, L. N. Thompson-Golden, T. J. Hackman, M. R. Ellersieck, L. Wax, D. P. Colling, J. B. Corners, and P. A. Lancaster. 2009. Effects of tasco in alleviation of heat stress in beef cattle. Prof. Anim. Sci. 25: 109-117.

Zhang, X. 1997. Influence of plant growth regulators on turfgrass growth, antioxidant status, and drought tolerance. Ph.D. dissertation. Virginia Polytechnic Institute and State Univ., Blacksburg.

Zimbelman, R. B., R. P. Rhoads, M. L. Rhoads, G. C. Duff, L. H. Baumgard, and R. J. Collier. 2006. A re-evaluation of the impact of temperature humidity index (THI) and black globe humidity index (BGHI) on milk production in high producing dairy cows. Available on line at http://animal.cals.arizona.edu/swnmc/Proceedings/2009/14Collier_09.pdf. Accessed May 04, 2010.