Departament de Ciència Animal i dels Aliments

Heat stress responses in dairy goats and effects of some nutritional strategies for mitigation

Respuesta de cabras lecheras al estrés térmico y efectos de algunas estrategias nutricionales para su mitigación

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- **Hamzaoui, S**., A.A.K. Salama, G. Caja, E. Albanell, C. Flores, and X. Such. 2010. Physiological and Nutritional changes of dairy goats for maintaining milk yield during extreme heat stress at late lactation. 61th EAAP Annual Meeting,Heraklion (Greece), Book of abstracts No. 16, p. 98.
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Annual Meeting, 2012. Phoenix (Arizona, USA), J. Dairy Sci. Vol. 95, (E-Suppl. 2): 684 (Abstr.).

- **S. Hamzaoui**, A.A.K. Salama, G. Caja, E. Albanell, X. Such. 2013. Supplementation with soybean oil increases milk fat and improves milk fatty acid profile in heat-stressed dairy goat. 2013. ADSA-ASAS Annual Meeting.Indiapolis (Indiana, USA), J. Dairy Sci. Vol. 96, (E-Suppl. 1): T358 (Abstr.).
- S. Hamzaoui, A.A.K. Salama, G. Caja, E. Albanell, X. Such. 2014. Supplementation with propylene glycol has no effect on milk protein content of heat-stressed dairy goat. ADSA-ASAS Annual Meeting.Kansas City (Missouri, USA), J. Dairy Sci. Vol. 97, (E-Suppl. 1): T258 (Abstr.).
- **S. Hamzaoui**, A.A.K. Salama, G. Caja, E. Albanell & X. Such. 2014. Milk fat content and fatty acid profile of heat-stressed dairy goats supplemented with soybean oil. 61th EAAP Annual Meeting,Copenhagen (Denmark), Book of abstracts No. 20, p. 403.

LIST OF ABREVIATIONS

AA	Amino-acid
ADF	Acid detergent fiber
ANOVA	Analyze of variance
bST	Bovine somatotropin
BHBA	Beta-hydroxybutyrate acid
BUN	Blood urine nitrogen
BW	Body weight
Cl^-	Chlorine
CLA	Conjugated linoleic acid
CN	Casein
CP	Crud protein
CO_2	Dioxide of carbon
pCO_2	Pressure CO_2
CVM	Complements of minerals and vitamins
DIM	Days in milking
DL	Data logger
DM	Dry matter
DMI	Dry matter intake
FA	Fatty acid
FCM 3.5%	Fat corrected milk 3.5%
GB	Gigabyte
GLM	General linear model
$HCO3^-$	Bicarbonate
HS	Heat stress
HSF1	Heat shock transcription factor 1
HSP	Heat shock proteins
H_2SO_4	Sulfuric acid
IGF-I	Insulin-like Growth factor I
IU	International units
kPa	Kilo Pascal
LCT	Lower critical temperature
Log10	Common logarithm
MBF	Mammary blood flow
Mcal	Mega calorie
MUFA	Monounsaturated fatty acids
NE_L	Net energy of lactation
NEFA	Non esterified fatty acids
NDF	Neutral detergent fiber
NPN	Non protein nitrogen

OM	Organic matter
pCO_2	Pressure of carbon dioxide
PCV	Packed cell volume
PDI	True protein digested in the small intestine
PDIA	Dietary protein undegraded in the rumen which is truly digestible in the small intestine
PDIE	True protein digested in the small intestine when fermentable energy is limiting
PDIFF	*P* difference
PDIN	True protein digestible in the small intestine when fermentable N is limiting
PG	Propylene glycol
r^2	Coefficient of determination of a linear regression
RDP	Ruminal degraded protein
RH	Relative humidity
RR	Respiration rate
RT	Rectal temperature
RUP	Ruminal undegradable protein
SBO	Soybean oil
SE	Standard error
SED	Standard error of deviation
SFA	Saturated fatty acids
SEM	Standard error of the mean
THI	Temperature humidity index
TMR	Total mixed ration
TN	Thermal neutral
TNZ	Thermoneutral zone
TS	Total solids
TVA	Trans vaccenic acid
TWI	Total water intake
T3	Triiodothyronine
T4	Thyroxine
UCT	Upper critical temperature
UEm	Fill unit system for sheep
UFL	Feed Unit system for milk
VFA	Volatile fatty acid

SUMMARY

In the current thesis 4 experiments were carried out using dairy goats under heat stress (HS) to measure responses to HS (Exp. 1 & 2) and to evaluate soybean oil and propylene glycol as feed supplements (Exp. 3 & 4). In Exp. 1 & 2, 8 Murciano-Granadina dairy goats in late (Exp. 1) and mid (Exp. 2) lactation were exposed to different ambient conditions, using metabolic cages in a climatic chamber. Experimental design was a crossover (2 periods of 28-35 d and 4 goats each), and conditions were: 1) thermal neutral (TN, 15 to 20°C day-night), and 2) heat stress (HS, 12-h day at 37°C and 12-h night at 30°C). Humidity was maintained at 40% and light-dark was constant (12-12 h). Rectal temperature and respiratory rate (0800, 1200 and 1700 h) and milk yield were recorded daily, whereas milk composition and blood parameters were evaluated weekly. Digestibility coefficients and N balance were determined and behavior was recorded by video cameras. Moreover, challenges with insulin (4.6 µg/kg BW), epinephrine (2 µg/kg BW) and glucose (0.25 g/kg BW) were done and blood samples were collected for the analysis insulin, NEFA and glucose concentrations. Compared to TN goats, HS goats experienced greater rectal temperature, respiratory rate, water intake, and water evaporation. Intake of HS goats decreased by 21 and 29% in Exp. 1 and 2, respectively. Milk of HS goats contained lower fat, protein and lactose. Panting reduced concentration and pressure of CO2 in blood of HS goats, but they were able to maintain their blood pH similar to TN group by lowering $HCO3^{-}$ in blood. The TN and HS goats had similar blood NEFA after insulin injection, but NEFA values were greater ($P < 0.05$) in TN than HS goats after epinephrine administration. The HS goats secreted lower ($P < 0.05$) amounts of insulin than TN goats in response to the glucose tolerance test. Furthermore, TN and HS goats had similar eating bouts, but the duration of each bout was lower in HS than in TN. On the other hand, HS had greater number of drinking bouts with no change in drinking bout durations.

In Exp. 3 & 4, 8 multiparous Murciano-Granadina dairy goats at mid lactation were used in a replicated 4 × 4 Latin square design with 4 periods; 21 d each (14 d adaptation, 5 d for measurements and 2 d transition between periods). Goats were allocated to one of 4 treatments in a 2 x 2 factorial arrangement. Factors were supplementation or not with soybean oil (Exp. 3) or propylene glycol (Exp.4, and TN or HS conditions similar to Exp. 1 & 2. Feed intake, milk yield, milk composition, and blood metabolites were evaluated. From the point of view of human health, HS improved milk fatty acid profile by decreasing saturated fatty acids and increasing monounsaturated fatty acids with no effect on milk fat content. The soybean oil increased ($P < 0.05$) on average blood NEFA by 50%, milk fat by 30%, and conjugated linoleic acid by 360%. The response to soybean oil was with the same magnitude in thermo-neutral and heat stress conditions. On the other hand, the supplementation with propylene glycol increased blood glucose ($P < 0.05$) and tended to increase ($P < 0.10$) blood insulin, but dry matter intake and milk fat decreased ($P < 0.10$). Furthermore, blood NEFA and β-hydroxybutyrate acid decreased ($P < 0.05$) by propylene glycol.

In conclusion, heat stress decreased milk yield by 3 to 10% with a marked reduction in milk protein. Lipid tissue of heat-stressed dairy goats became insensitive to lipolytic hormones and their pancreas secreted lower insulin when glucose was injected. Heat stress had no effect on eating bouts, but the time of each eating bout was shorter. The supplementation with soybean oil increased milk fat, trans-vaccenic acid and conjugated linoleic acid similarly in thermo-neutral as well as in heat stress conditions. Although propylene glycol increased blood glucose and insulin, no change in milk protein was observed.

RESUMEN

En la presente tesis se han llevado a cabo 4 experimentos con cabras lecheras bajo condiciones de estrés por calor (HS) para medir la respuesta bajo las condiciones de estrés (Exp. 1 y 2) y para evaluar el aceite de soja y el propilenglicol como suplementos alimenticios (Exp. 3 y 4). En los Exp. 1 y 2, 8 cabras lecheras de raza Murciano-Granadina, a final (Exp. 1) y a mitad (Exp. 2) de lactación fueron expuestas a diferentes condiciones ambientales, utilizando jaulas metabólicas en una cámara climática. El diseño experimental fue de efecto cruzado (2 periodos de 28-35 d y 4 cabras por grupo) y las condiciones fueron: 1) temperatura neutral (TN, 15 a 20ºC día-noche) y 2) estrés por calor (HS, 12-h d a 37ºC y 12-h noche a 30ºC). La humedad se mantuvo al 40% y luz-oscuridad fue constante (12-12h). Diariamente, se midió la temperatura rectal, la frecuencia respiratoria (0800, 1200 and 1700 h) y la producción de leche, mientras que la composición de la leche y los parámetros sanguíneos fueron evaluados semanalmente. Se determinó los coeficientes de digestibilidad y el balance N y se registró el comportamiento mediante cámaras de video. Además, se realizaron tratamientos con insulina (4.6 µg/kg BW), epinefrina (2 µg/kg BW) y glucosa (0.25 g/kg BW) y se tomaron muestras de sangre para analizar insulina, NEFA y concentraciones de glucosa. En comparación con las cabras TN, las cabras HS experimentaron una mayor temperatura rectal, frecuencia respiratoria, consumo de agua y evaporación de agua. La ingesta de las cabras HS decreció un 21 y 29% en los Exp. 1 i 2, respectivamente. La leche de las cabras HS mostraron un menor porcentaje de grasa, proteína y lactosa. En comparación a las cabras TN, las cabras HS disminuyeron la concentración y la presión sanguínea del CO_2 debido al jadeo y mantuvieron el pH sanguineo al bajar la concentración de HCO_3^-. Las cabras TN y HS tuvieron niveles similares de NEFA en sangre después de la inyección de insulina, pero después de la administración de epinefrina los valores de NEFA fueron mayores ($P < 0.05$) en las cabras TN que en las HS. Las cabras HS secretaron menos ($P < 0.05$) insulina que las cabras TN en respuesta al test de tolerancia de glucosa. Las cabras TN y HS presentaron una similar frecuencia alimentaria, aunque la duración de cada ingesta fue menor en las cabras HS que en las TN. Por otra lado, las cabras HS tuvieron una mayor frecuencia de bebida, aunque no hubo variación en la duración.

En los Exp. 3 i 4, se utilizaron 8 cabras lecheras multíparas a mitad de lactación de raza Murciano-Granadinas en un diseño de cuadrado latino 4 x 4 con 4 periodos de 21 d cada uno (14 d de adaptación, 5 d de medidas y 2 d de transición entre periodos). Las cabras fueron asignadas a 4 grupos con un diseño factorial 2 x 2. Los factores fueron la suplementación o no suplementación con aceite de soja (Exp. 3) y propilenglicol (Exp. 4) en condiciones de TN o HS iguales a lo mencionado en Exp. 1 y 2. Se evaluó la ingestión, la producción lechera, la composición de la leche y los metabolitos sanguíneos. Desde el punto de vista de salud humana, el HS mejoró el perfil de ácidos grasos de la leche debido a la disminución de los ácidos grasos saturados y el aumento de los ácidos grasos monoinsaturados sin afectar la grasa. El aceite de soja incrementó ($P < 0.05$) las NEFA en sangre en un 50%, la grasa de la leche en un 30% y el ácido linoleico conjugado en un 360%. La respuesta al aceite de soja fue de la misma magnitud en cabras TN y HS. Por el otro lado, la suplementación con propilenglicol aumentó los niveles de glucosa ($P < 0.05$) e insulina ($P < 0.10$), pero disminuyó ($P < 0.10$) la ingestión y la grasa en leche. Además, los niveles de NEFA y BHBA fueron menores en las cabras suplementadas con el propilenglicol.

En conclusion, el HS disminuyó la producción lechera entre un 3 y 10% con una marcada reducción en la proteína de la leche. El tejido lipídico de las cabras HS se volvió insensible a las hormonas lipolíticas, secretando el páncreas menor cantidad de insulina al inyectarle glucosa. El HS no afectó el número de acercamientos al comedero, pero sí redujo su duración. La suplementación con aceite de soja en condiciones HS y TN incrementó de forma similar la grasa de la leche, el ácido trans-vacénico y el ácido linoleico conjugado. Finalmente, el

propienglicol incrementó el nivel de glucose e insulina sanguíneo, pero no alteró la proteína de la leche.

TABLE OF CONTENTS

CHAPTER 1

Literature review

CHAPTER 1

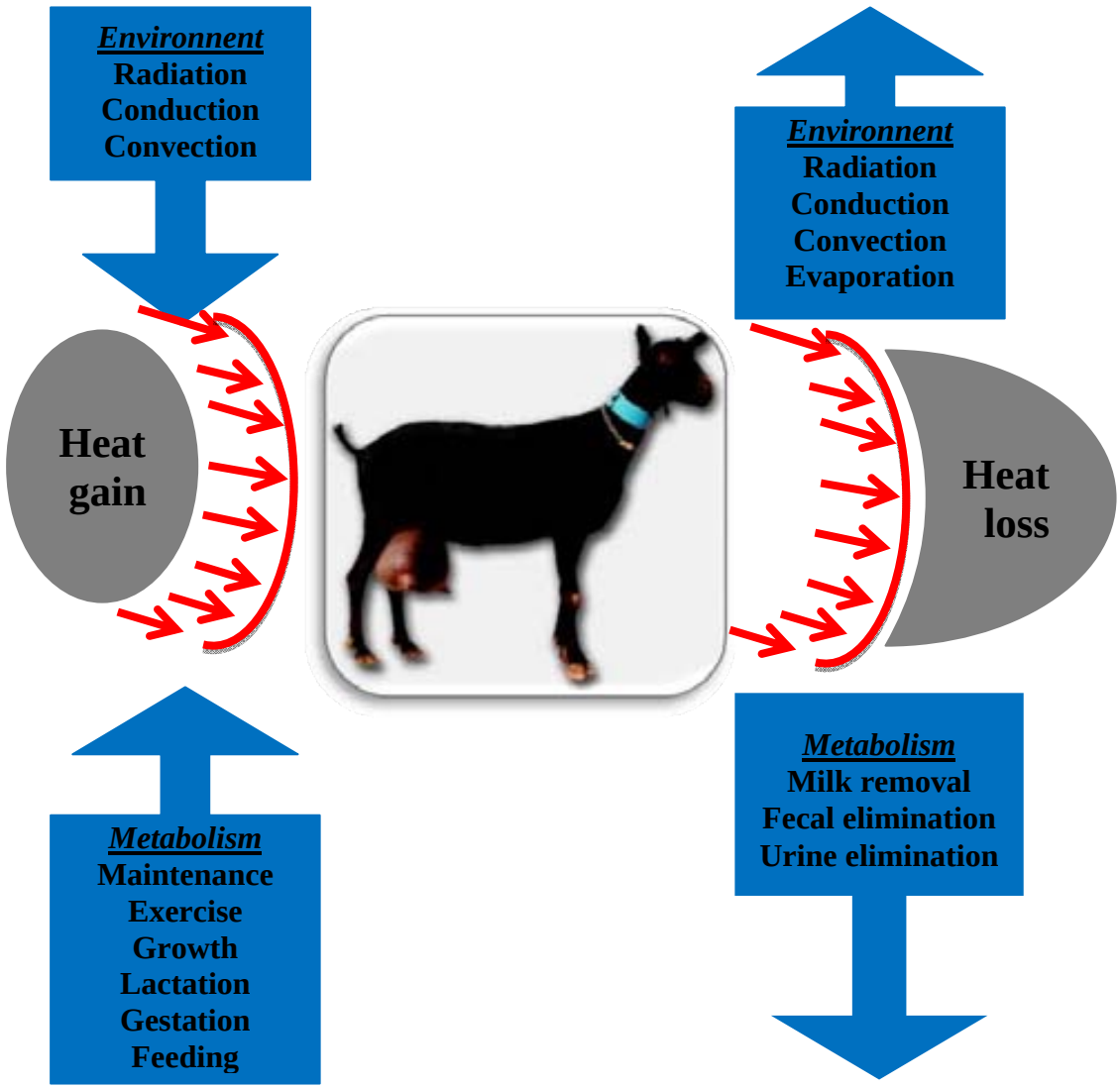
LITERATURE REVIEW

1.1 Thermoregulation in ruminants

Like any homeothermic animal, a ruminant needs to maintain its body temperature within a very narrow interval, whatever its own heat production and the environmental conditions. Maintaining the body temperature is necessary for optimal physiological functions and cellular metabolic reactions. Thermoregulation is the process by which an animal maintains its body temperature constant by the balance between heat gain and heat loss (Figure 1.1). According to Lee (1965), heat load on the animal is affected by numerous factors including: (a) environmental variables: temperature, humidity, air movement, radiation, and precipitation; and (b) animal characteristics: species, age, sex, breed, metabolic state, coat, acclimatization, nutrition, disease, and individual variability. These many factors make it complex to describe and predict the impact of environment on dairy animals.

The animal could transform an important part of the energy intake to heat production. Coppock (1985) indicated that heat production, resulting from metabolic functions, accounts for approximately 31% of energy intake by a 600-kg cow producing 40 kg of milk containing 4% fat. The heat production accumulated by the animal (heat gain) subjected to heat stress (HS) is the sum of heat accumulated from the environment and the failure to dissipate heat associated with metabolic processes. In this regard, under HS conditions, the animal should loss heat to maintain its body temperature. It is well known that outside the thermoneutral zone (TNZ), the animal is stressed (cold or heat) and an extra energy for maintenance is necessary. The NRC (1981) reported that the maintenance expenditures at 35°C increased by 20% over thermoneutral conditions, thus increasing the cow's energy expenditure, often at the expense of milk yield. Generally, the TNZ range depends on age, species, breed, feed intake, diet composition, previous state of temperature acclimation, production level, housing and pen conditions, coat type and color, and behavior (Yousef, 1985).

Figure 1.1. Thermoregulation in ruminants by the balance between heat gain and heat loss.



Obviously with similar body size and surface area, the lactating cow has significantly more heat to dissipate than a nonlactating cow and will have greater difficulty dissipating the heat during hot and humid conditions. Purwanto et al. (1990) found that low (18.5 kg/d) and high (31.6 kg/d) yielding cows generated 27 and 48% more heat than nonlactating cows despite having lower BW (752, 624, and 597 kg for nonlactating, low, and high producers, respectively). Moreover, heat production was greater for cows administered bovine Somatotropin (bST) compared with controls in a hot humid climate, with low yielding cows more responsive to bST than high yielding cows (Manalu et al., 1991).

1.2. Heat dissipation mechanisms: interaction with animal characteristics

As shown in figure 1.1, heat can be dissipated by radiation, conduction, convection, evaporation. As the ambient temperature rises, heat dissipation is shifted from radiation, conduction and convection to evaporation. However, animals are not able to cool themselves (without artificial evaporative cooling) and maintain their physiological functions when the environmental heat load exceeds a specific upper critical temperature. Appleman and Delouche (1958) observed that the heat regulatory system of Nubian goats was no longer effective when animals were continuously kept for 12 consecutive days at 40º C, but not at 35ºC. Consequently, they concluded that the limit of heat tolerance for goats lies between 35 and 40º C.

Radiation would be defined by the amount of radiant heat absorbed by an object. It depends not only on the temperature of the object, but also on its color and texture, with dark surfaces radiating and absorbing more heat than light colored surfaces at the same temperature. An animal with a black coat will, therefore, have an absorbance of 1; whereas, a white-coated one will have an absorbance of 0.37 and one with red fur has an absorbance of 0.65 (Cena and Monteith, 1975). In practical conditions, provision of shade, even by simplest means, is the primary means for reduction of incoming sun radiation. Provision of shade would reduce radiant heat load from the upper hemisphere by 30 to 70%, depending on shade quality.

According to Finch et al. (1980), black goats are dominant in hot deserts and have advantages for solar radiation exposure over white goats. Although the black coat absorbs much more solar radiation, black goats are able to look for food for longer time under sun. Black goats drink an amount of water equal to about 35% of their body weight and are able to efficiently cool themselves by evaporation.

Short haired goats exposed to solar radiation had greater increases in rectal and dermal temperatures, respiratory and pulse rates, and consumed less feed than the long haired goats (Acharya et al., 1995). Those authors concluded that long haired goats tolerated radiant heat better than short haired goats. Moreover, cows have an apocrine sweat gland with one sweat gland associated with each hair fiber. Consequently, hair density directly reflects the number of sweat glands, and hair diameter and length have effects on evaporative heat loss by regulating airflow at the skin surface (Collier et al., 2008). Those authors showed the existence of a single gene affecting hair coat density and hair length. Cows bearing that gene

have a greater ability to deliver heat from the body core to the skin (evaporative heat loss) under HS conditions.

When cool air comes in contact with a warm body, a layer of air surrounding the surface of the body is heated and rises moving away from the body, carrying with it heat, and thereby cooling the body through the process of convection. On the contrary, if air temperature is greater than skin temperature, then air movement will promote the movement of heat into the animal until air temperature equals skin temperature when transfer of heat ceases (Kadzere et al., 2002).

Kadzere et al. (2002) defined conductive heat exchange as heat flow between two media or bodies in direct contact (e.g. contact between animal body and the ground). The magnitude of conductive heat transfer by conduction depends on the temperature difference, thickness of the conducting materials, and the surface area of contact and thermal conductivity of the conducting objects (Schmidt-Nielsen, 1964; Spiers, 2012).

Under HS conditions, utilization of bedding materials with high conductance may facilitate cooling of the animals. From experiments with different bedding materials (wood shavings, sand, ground limestone, shredded paper and rubber mats), Cummins (1998) found that cows had highest preference for ground limestone which had the lowest temperature of 25.9 ºC at 25 mm below the surface. This underscores the importance of bedding material selection as part of heat stress abatement strategies. In the standing animal, conductive heat loss is minimal because of the presence of a layer of air against the skin, which means that most of the heat transfer from the animal takes place to air, and air has a poor thermal conductivity (Yousef, 1985).

Evaporative heat loss involves cutaneous (sweating) and water loss through the respiration (panting). Sweating leads to evaporative heat loss from the skin surface, whereas in panting, sensible heat is used to heat the water vapor and remove heat in the form of vaporized moisture from the lungs (Gebremedhin, 2012). Evaporative cooling from the outer surface is the most effective mechanism of heat dissipation under hot dry conditions. Khelil-Arfa et al. (2014) described that the first heat adaptation mechanism of homoeothermic animals is the increase in water loss by evaporation.

Heat loss by cutaneous evaporation accounts for 20 to 30% of the total heat loss when air temperature is between 10 and 20ºC, but when air temperature is greater than 30ºC, cutaneous evaporation becomes the primary way for heat loss, accounting for approximately 80 to 85% of the total heat loss, while the rest is lost by respiratory evaporation (Silva et al., 2009; Maia et al., 2005a,b). Appleman and Delouche (1958) observed a strong relationship between

respiratory rate and water consumption in goats. As respiratory rate increased and more water was vaporized, more water was consumed (Gebremedhin, chapter 3, 2012). Consequently, a dramatic increase in water intake (+77%) and water evaporation (+207%) was observed in dairy goats (Hamzaoui et al., 2013a).

Evaporative cooling is affected by wind velocity, relative humidity, and thermal and solar radiation. Other factors that affect the efficacy of evaporative cooling from the skin surface are hair coat density and thickness, hair length and color, and skin color as previously mentioned. The air velocity remains the most important factor influencing evaporative heat transfer of the skin surface by sweating. Thereby, the common method of cooling animals is to blow air over them. Wind penetrates the hair coat and reduces the effective thermal resistance of the hair coat (McArthur, 1987; Berman, 2004). Thermal resistance of the hair coat decreases linearly with increasing air velocity. Hillman et al. (2001b) reported that increasing air velocity over a dairy cow from 0.2 to 0.9 m/s increased sweating rate from 75 to 350 g/m2/h. These findings were pivotal to the development of modern forms of air ventilation to ameliorate heat stress in domestic animals.

1.3. Responses to heat stress in ruminants

1.3.1. Physiological responses

1.3.1.1. Body temperature

The rectal temperature (RT) is an indicator of thermal balance and may be used to assess the adversity of the thermal environment (Johnson, 1980). A rise of 1ºC or less in RT is enough to reduce performance in most livestock species (McDowell et al., 1976). It appears that there are notable differences between breeds in their abilities to regulate RT; the mean RT is higher in Bos taurus than in Bos indicus cattle (Finch, 1986) and, as a result, Bos taurus cattle are more sensitive to HS than their Bos indicus counterparts.

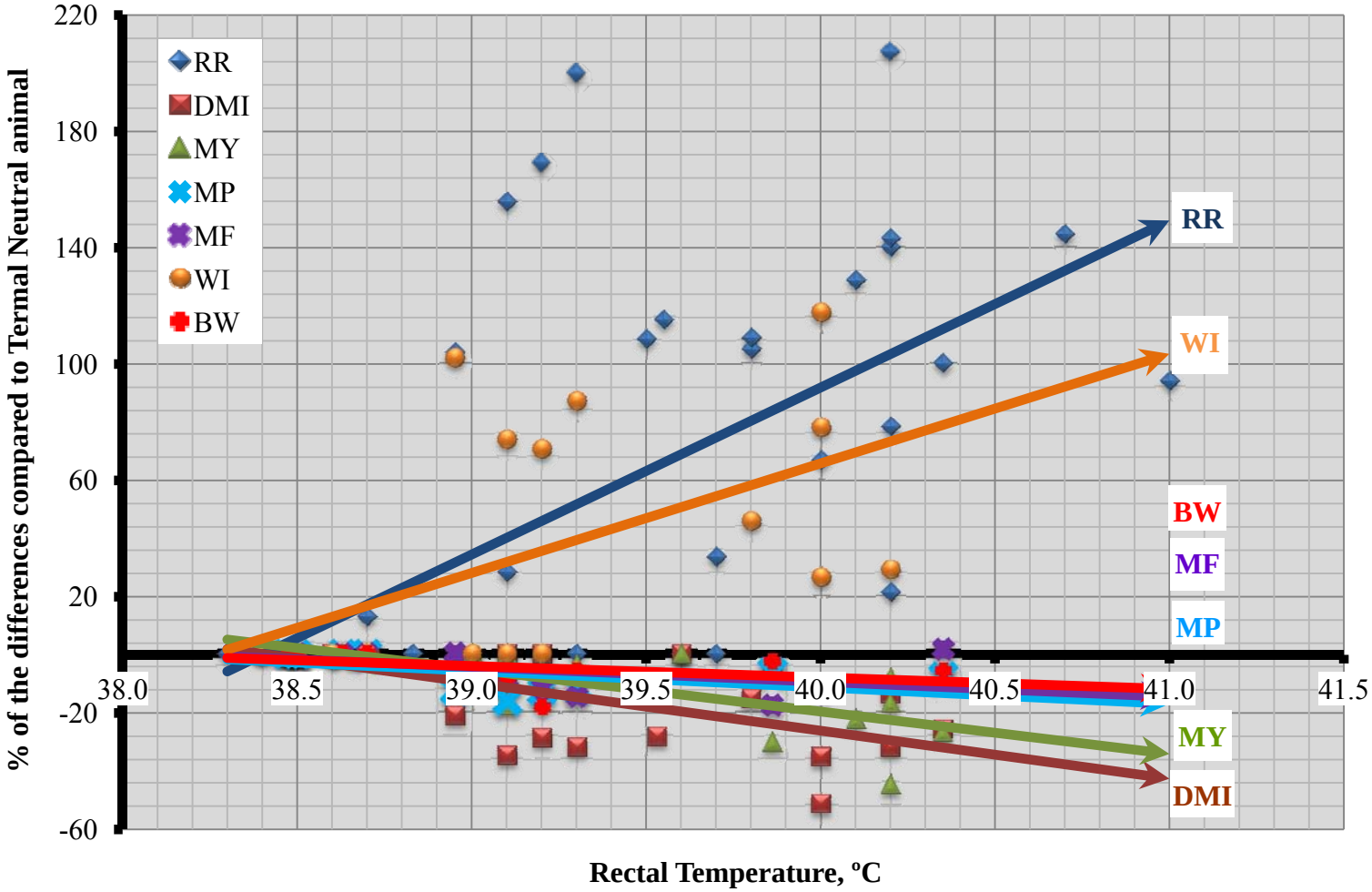
As shown in Figure 1.2, we reviewed 20 papers to find the relationship between the variation of RT and other performance parameters [respiration rate (RR), water intake (WI), DMI, milk yield (MY), milk protein (MP), milk fat (MF), and body weight (BW)] in cows, sheep and goats under HS conditions. The response in RR was the highest and fastest to the increase in RT, followed by the increment in the WI under HS conditions. Decreases in DMI, MY, MP, MF and BW were also observed. Physiologically, it is logical that the animal first dissipates the heat loaded by respiration, which needs more water consumption, and if the HS

persists, the animal decreases the DMI to reduce the heat production. Consequently, animal losses weight and milk production (yield and composition) is depressed.

Hillman et al. (2005) reported that RT is a more reliable indicator of HS than respiration rate or skin temperature. However, RT measurement has some disadvantages, among them, the use of a rectal probe can disturb the behavior of the animal, and the procedure is time consuming, and more importantly, does not provide a continuous record. Moreover, Burfeind (2010) reported in dairy cows that RT measures could be influenced by type of thermometer used (up to 0.3°C difference), and the penetration depth into the rectum (up to 0.4°C difference). Besides the RT, there are different temperatures that may represent the internal body temperature including skin, tympanic membrane, and vaginal temperatures. Generally, there was a high positive relationship (R^2 = 0.77 to 95) among different types of body temperature measurements in steers (Hahn et al., 1990) and cows (Rajamahendran et al., 1989; Kennedy, 2000; Hillman et al.. 2009).

An alternative approach is the continuous measurements of vaginal temperature by data loggers (Collier et al., 2006). Vaginal temperatures were associated with RT, and provided the advantage of capturing diurnal changes in body temperature (Vickers et al., 2010). Skin temperature is affected by physiological and thermal factors jointly with physical and optical properties of hair coat. Recently, use of infrared thermography guns has been shown to be a low-cost approach to measure skin surface temperature of animals. If the skin surface temperature is below 35°C, the temperature gradient between the core and skin is large enough for the animals to effectively use all 4 routes of heat exchange. Infrared skin temperature is highly correlated with respiration rates and is a good measure of the microenvironment around the animal. Furthermore, the measurement can be taken from a distance, which does not require restricting movement of the animals (Collier et al., 2006).

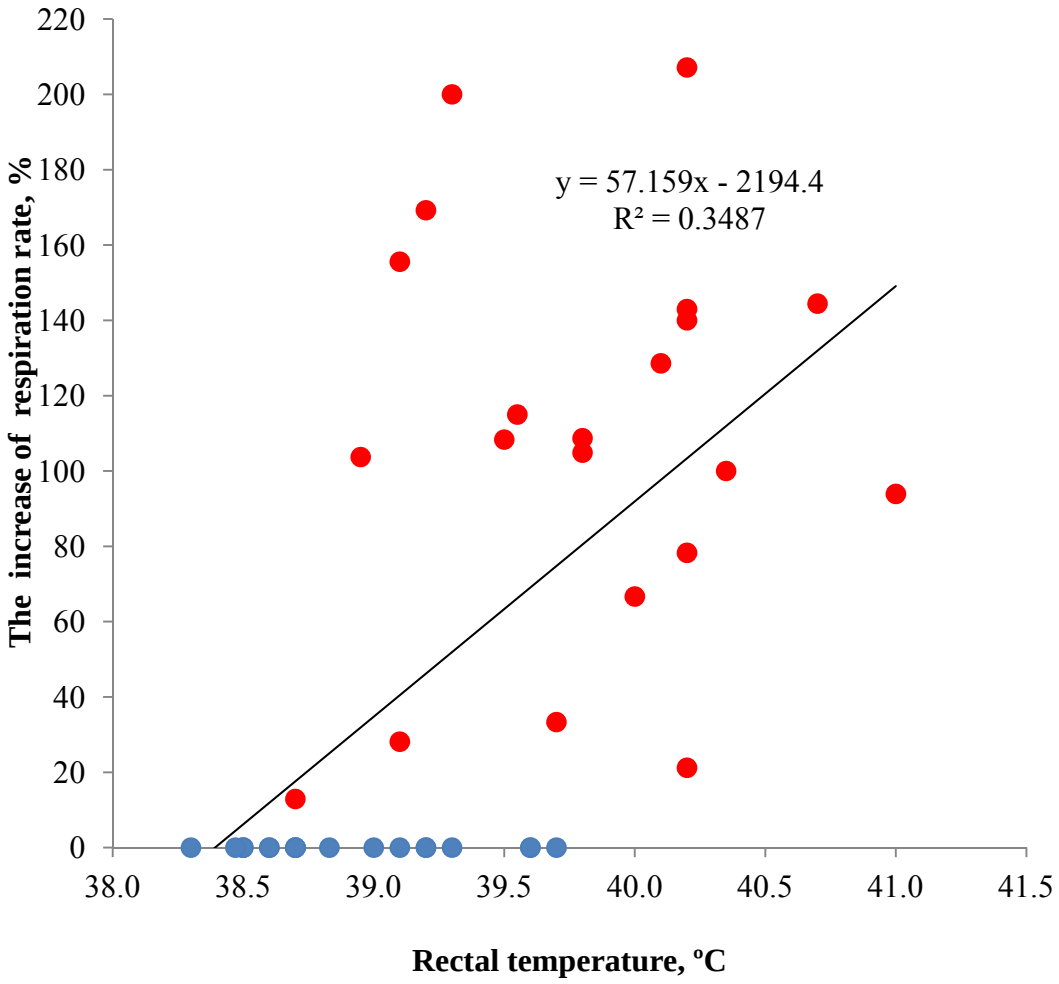
Figure 1.2. The effect of the rectal temperature on some performance parameters of ruminants (cows, sheep, and goats) under heat stress compared to thermal neutral conditions. Data pointes were extracted form 22 papers. Performance parameters were (RR: resperation rate; DMI: dry matter intake; MY: milk yield; MP: milk protein; MF: milk fat; WI: water intake and BW: body weight.



1.3.1.2. Respiration rate

As above indicated, evaporation (by respiration and sweating) is an important via for heat loss under high ambient temperatures. Thompson, (1985) estimated the heat loss in sheep by approximately 20% of total body heat via respiratory moisture in a neutral environmental temperature (12ºC). However, the moisture loss increases and accounts for approximately 60% of the total heat loss at high ambient temperature (35ºC). Consequently, RR dramatically increased under HS by up to 200% (Figure 1.3). As shown in Figure 1.3, animals start to have high RR from 39ºC of body temperature and the majority of RR rises within the interval of 2ºC (39 to 41ºC).

Figure 1.3: Relationship of rectal temperature with water consumption (% of change with regard to the control) in cows, sheep, and goats under heat stress compared to thermal neutral conditions. Data pointes were extracted from 22 papers. Blue points indicate the control values, whereas red points indicate the response under HS.



1.3.1.3. Pulse rate (PR)

Generally, in the wide range of environmental temperatures that comprise the thermo-neutral zone, animals maintain their heat balance via vasomotor control by regulating the amount of blood flowing through the cutaneous vessel, by either vasodilatation or vasoconstriction. Vasodilatation stimulates the pilomotor center to flatten the hair cover to allow better heat dissipation through non-evaporative and evaporative mechanisms. As the ambient temperature increases, the pulse rate (PR), as well as, the circulation of blood increases to transfer heat from the core to the periphery (Aboul-Naga, 1987; Marai et al., 2007). Therefore, during summer the PR is significantly higher than during winter (Khan and Ghosh, 1989; Abi-Saab and Saleim, 1995; Ismail et al., 1995; Alexiev et al., 2004).

Nevertheless, at very high temperatures the PR may decrease due to a decrease in the metabolic rate in cows (Kibler and Brody, 1951; Bianca, 1959; Singh and Newton, 1978; Richards, 1985) and in sheep (Sakurada and Hales, 1998; Marai et al., 2007). Hence, a reduced PR is more typical in heat-stressed animals as it is associated with a reduced rate of heat production as a response to high environmental temperatures. However, Huhnke and Monty (1976) did not find any significant variation in the PR due to variation in ambient temperature. Muller and Botha (1993) concluded that PR was not influenced to the same extent as RT and respiratory rate (RR) by increasing ambient temperatures, and therefore, it is not a reliable indicator for HS.

1.3.1.4. Sweating rate

There are two types of sweating that are appreciably involved in heat dissipation. The first type is insensible sweating or perspiration that leaves the body at all times, unless the relative humidity is 100%. Another type, thermal sweating, occurs as the principle evaporative cooling mechanism of the animal when ambient temperatures rise. However, animals have different densities of sweat glands per unit area with one per each hair follicle (Findlay and Yang, 1950). According to Dowling (1955), Bos indicus breeds have a higher density of hair follicles (1698 /cm for Zebu) than for Bos aurus breeds (1064 /cm for Shorthorn), and that makes the differences in the secretion of sweat between breeds.

Electrolyte status is altered by the exposure of animals to hot weather (Shalit et al., 1991; Collier et al., 1982; Schneider et al., 1988). Shalit et al. (1991) found that water turnover and the output of Na, K, and Cl in milk and sweat increase markedly under hot conditions. The K is the main cation in bovine sweat, and under HS secretion of K through sweat increases,

resulting in lower K concentration in blood (Johnson, 1970; El-Nouty et al. 1980; Beede et al., 1983; Mallonee et al., 1985). Greater losses of K in sweating cause an increment in K requirements by as much as 12% (Collier et al., 2006). Moreover, Jenkinson and Mabon, (1973) noted marked increases in the rates of loss of Na, Mg, Ca, and Cl, but not P, and the authors found significant correlations between losses and sweating rates. Similarly, Kume et al. (1987; 1989) reported that trace element requirements may increase with elevated environmental temperatures.

1.3.2. Behavior changes

Domesticated ruminants are diurnal in nature, being active during the day and resting at night (Silanikove, 2000). During hot weather, this behavior shifts. Distressed animals may seek shade, change its orientation to the sun (decreasing exposure to solar load), and increase water intake (Blackshaw and Blackshaw, 1994; Hamzaoui et al., 2013a). Moreover, grazing occurs before sunrise, at dawn, and during the night. The animals tend to lie down and decrease their locomotion during the day. Other adaptation that may occurs; the animal moistens its body with water, saliva, or nose secretions to increase heat loss by convection and conduction. Reducing feed intake is also a kind of autonomic thermoregulatory behavior to decrease heat production. In dairy cows, Schneider et al. (1988) and Nardone et al. (1992) found that heat-stressed cows changed their feeding behavior. In particular, heat-stressed cows ate 12% more during the nighttime, when temperatures were cooler, compared with daytime. Under heat stress conditions cattle reduce roughage intake (Collier et al., 1982; Coppock et al., 1986; Bernabucci et al., 1999). This leads to changes in the forage-to-concentrate ratio of the diet ingested (Bernabucci et al., 1999). Cows in thermal neutral conditions typically consume 12 to 15 meals per day but decrease eating frequency to 3 to 5 meals per day during heat stress. The decreased frequency is accompanied by larger meals and thus more acid production post-eating. Furthermore, cows will typically gorge (over-eat) the day following a heat wave and this gluttonous behavior is well known to cause rumen acidosis (Bernabucchi, 2012).

1.4 Productive performances under heat stress

The HS animals experience a dramatic increase in water consumption (Figure 1.5), which is useful because the high specific heat of water allows the animal to absorb a big amount of heat during the day and dissipate it during the cool night. Moreover, HS reduced feed intake

(Figure 1.6) and in the same time increased maintenance requirements necessary for extra activities (e.g. muscle movements for panting, greater sweating, increased chemical reactions in the body, and the production of heat shock proteins that consumes large amounts of ATP). According to NRC (2007), there is a 30% increase in maintenance requirements under HS conditions. Consequently, energy intake would not be enough to cover the daily requirements for milk production, especially in early lactating animals.

Studies carried out in the University of Arizona using the pair-fed model demonstrated that reduced feed intake explains only 35 to 50% of the decreased milk yield during environmental-induced hyperthermia in dairy cows (Baumgard and Rhoads, 2013). In case of dairy goats under HS, it is unknown how much of the decrease in milk yield is due to reduced feed intake. However, dairy goats with feed restriction (-35% DMI) and supplemented with 1,3-butanediol had lower blood insulin, greater blood NEFA, and produced -22% lower milk yield than goats without feed restriction (Drackley et al., 1989). It seems that reduced feed intake could explain all of the milk yield losses under HS in dairy goats, but this assumption should be confirmed by pair-fed experiments in dairy goats.

Figure 1.5: The effect of the temperature humidity index (THI) on the increase of water intake (%) of ruminants under heat stress compared to thermal neutral conditions.

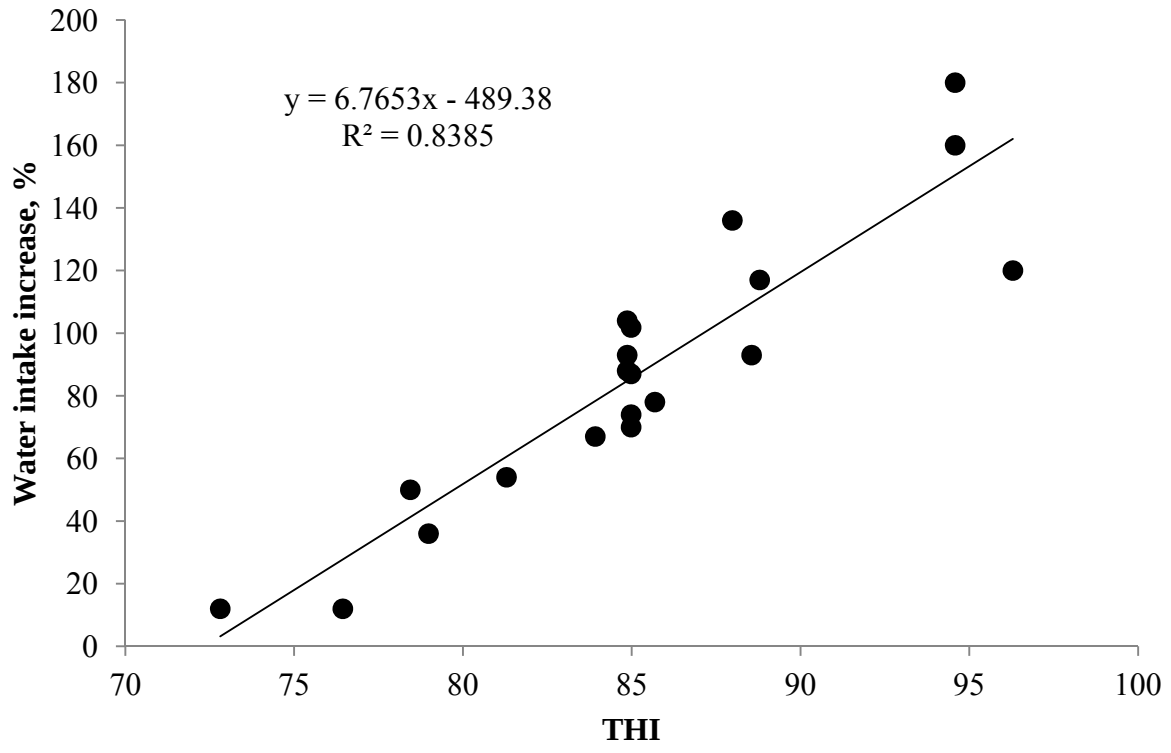
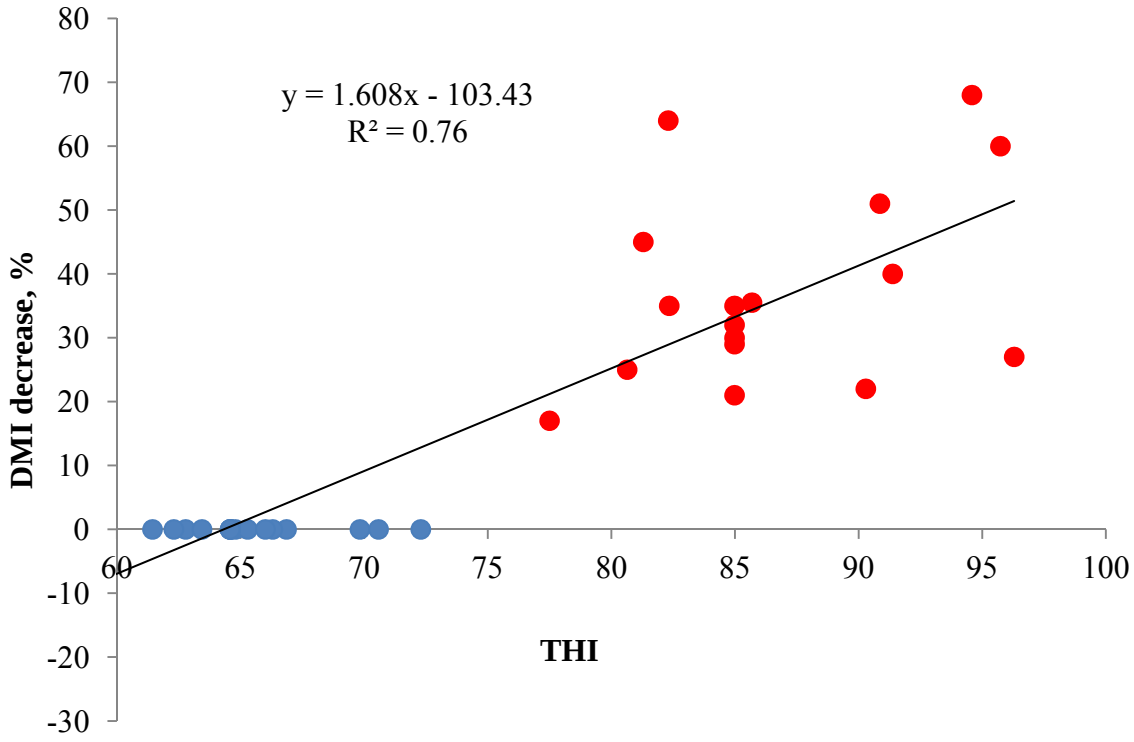


Figure 1.6. The effect of the temperature humidity index (THI) on the decrease of DMI (%) of cows, sheep and goats under heat stress compared to thermal neutral conditions. Data pointes were extracted from 22 papers. Blue points indicate the control values, whereas red points indicate the response under HS.

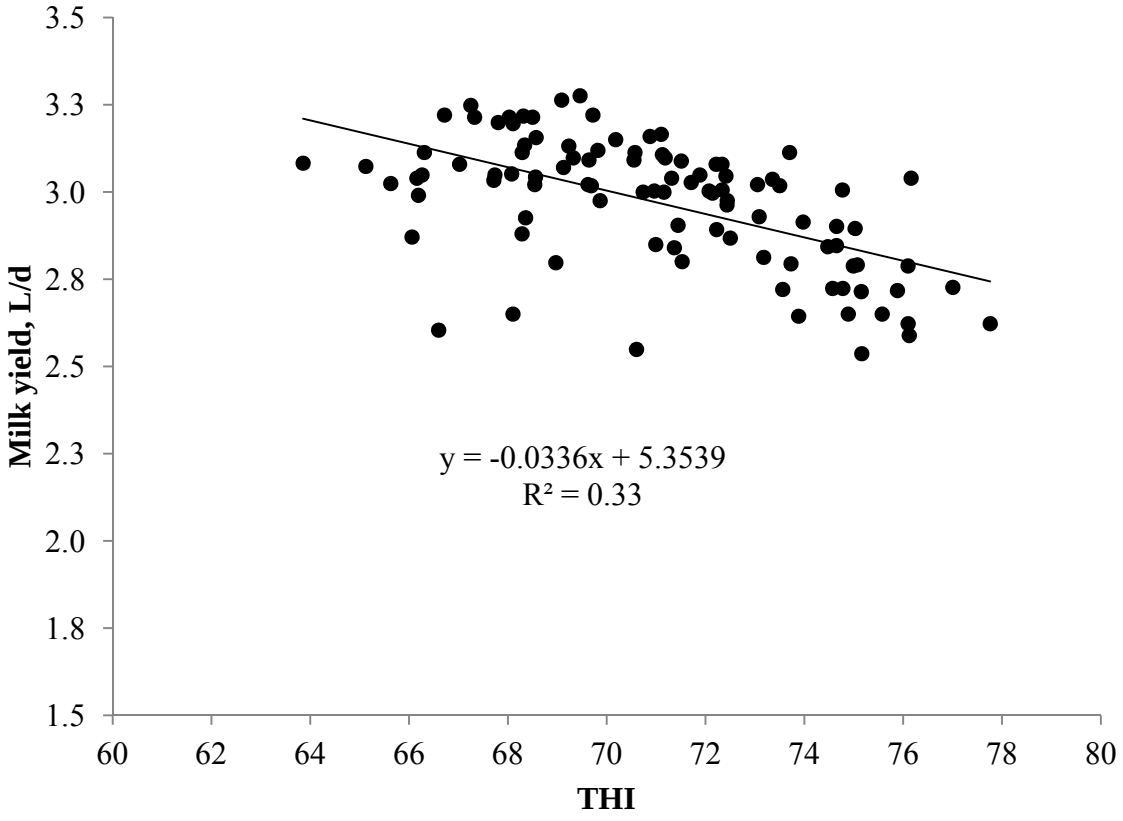


Dairy Saanen goats exposed to moderate or severe HS for 4 days (THI, 81 or 89) lost milk yield by 3 or 13%, respectively (Sano et al., 1985). Brown et al. (1988) reported that the exposure of dairy goats to moderate HS conditions for 5 weeks (34ºC and 25% humidity; THI = 79) depressed milk yield in Alpine but not in Nubian goats, indicating that the response to HS varies according to breed. As shown in Figure 1.4, milk yield in dairy goats decreased as THI value increased, and for each 1 unit increment of THI there is a decrease of 1% in milk yield. Although feed intake decreased similarly (-22 to -35%) in dairy goats and cows by environmental-induced HS, milk yield losses in dairy goats (-3 to -13%) were much lower than values reported in dairy cows (-27 to -33%) (Rhoads et al., 2009; Shwartz et al., 2009; Wheelock et al., 2010).

Losses in milk yield could vary according to the point on the lactation curve at which the animal experiences HS. Studies carried out in climatic chambers described a heat stress-related decrease in milk yield of 35% in mid-lactating (Nardone, et al. 1992) and of 14% in

early-lactating dairy cows (Lacetera et al., 1996). Variation of nutritional-metabolic conditions during lactation might explain the higher sensitivity to high ambient temperature of early- and mid-lactating dairy cows.

Figure 1.4. Relationship between temperature humidity index (THI) and milk yield in dairy goats (Salama et al., 2014).



The HS goats produced milk with lower protein (-6 to -13%) and lactose (-1 to -5%) contents (Brasil et al., 2000; Hamzaoui et al., 2013a). Increased sweat secretion that contains protein and urea (Joshi et al., 1968) together with decreased protein intake under HS might have limited the availability of amino acids for milk protein synthesis.

Heat-stressed ruminants consume less feed and consequently ruminate less (Aganga et al., 1990), resulting in decreased buffering agents entering the rumen. In addition, because of the redistribution of blood flow to the periphery (in an attempt to enhance heat dissipation) and subsequent reduction in blood delivery to the gastrointestinal track, VFA are absorbed less efficiently and thus the rumen VFA accumulate (and pH decreases). Furthermore, increased respiration rates also contribute to rumen acidosis because panting causes enhanced CO2 to be exhaled. In order to be an effective blood–pH buffering system, the body needs to maintain

a 20:1 HCO^3- (bicarbonate) to CO_2 ratio. Because of the hyperventilation-induced decrease in blood CO_2, the kidney secretes HCO^3- to maintain this ratio. This reduces the amount of HCO^3- that can be used (via saliva) to buffer and maintain a healthy rumen pH (Kadzere et al., 2002).

The HS resulted in greater values of digestibility of DM, CP, NDF, and ADF (+3 to +9 points) in dairy goats (Hamzaoui et al., 2013), male goats (Hirayama et al., 2004), dairy cows (McDowell et al., 1969), and heifers (Bernabucci et al., 1999). The increased digestibility under HS treatments might be partially due to the reduction of feed intake. Another reason for the enhanced digestibility under HS conditions could be a depressed passage rate of the solid phase of digesta as reported by Bernabucci et al. (1999).

Bernabucci et al. (2002) showed that milk produced from cows in summer contained lower concentrations of αs-CN and β-CN, which might explain the alteration in cheese-making properties of milk commonly observed during the summer. According to Abdel-Gawad et al. (2012), milk from HS goats had unexpected behavior during the curd firming stage of coagulation, which would have a negative impact on cheese making process control operations. The reasons for this altered firming behavior and the impact that would have on cheese production is still needed to be elucidated.

1.5 Gene expression regulation under heat stress

In dairy cows, Collier et al. (2008) reviewed the genes involved in the bovine HS response and concluded that gene networks within and across cells and tissues respond to environmental heat loads above the TNZ with both intra- and extracellular signals that coordinate cellular and whole-animal metabolism. Activation of these systems appears to be initiated at skin surface temperatures exceeding 35°C as animals begin to store heat and rapidly increase cutaneous evaporative heat loss (EVHL) mechanisms. Those authors summarized that gene expression changes include:

1. Activation of heat shock transcription factor 1 (HSF1);
2. Increased expression of heat shock proteins (HSP) and decreased expression and synthesis of other proteins;
3. Increased glucose and amino acid oxidation and reduced fatty acid metabolism;
4. Endocrine system activation of the stress response;
5. Immune system activation via extracellular secretion of HSP.

If the stress persists, these gene expression changes lead to an altered physiological state referred to as acclimation, a process largely controlled by altered gene expression in response to endocrine signals. In the acclimated state, metabolism is adjusted to minimize detrimental effects of increased thermal heat load. The variation in EVHL among animals and the central role that HSF1 has in coordinating thermal tolerance suggest that there is opportunity to improve thermotolerance via manipulation of the genes controlling expression of HSF1 and those regulating EVHL in cattle. Determining the basis for altered energy metabolism during thermal stress will lead to opportunities for improved animal performance via altered nutritional management.

Salama et al. (2014) used Microarrays of blood cells and RNA sequencing (RNA-seq) of milk cells to study changes in the gene expression of dairy goats under HS conditions. They identified 39 and 74 genes whose expression was up- and down-regulated, respectively by HS in the blood cells of dairy goats. The pathways affected by HS were related to cell proliferation and death, free radical scavenging, inflammatory response, and glycolysis/gluconeogenesis. The most transcription regulators affected by HS in blood cells were SATB1 (global chromatin organizer) and PPARD (peroxisome proliferator-activated receptor related to lipid metabolism). It seems that the functions of blood immune cells are altered due to changes in their lipid metabolism. This altered functionality of immune cells could increase the susceptibility of HS goats to infections. Testing the response of HS animals to challenges with pathogens needs further investigation.

As milk contains viable mammary epithelial cells, gene expression in milk cells reflects the transcriptional status of the mammary gland as affected by different nutritional, hormonal, and pathologic status (Boutinaud et al., 2002). Moreover, most of the genes expressed in the mammary gland transcriptome were present in milk somatic cells (Wickramasinghe et al., 2012). Consequently, they considered this approach appropriate over mammary biopsies due to the heterogenecity of gene expression in the mammary tissue (Molenaar et al., 1992). Thus, local biopsies not reflect gene dynamics in the whole mammary gland. The RNA was extracted from milk and RNA-seq was carried out in order to investigate whether changes in milk components by HS (e.g. lower contents of fat, protein and lactose) were accompanied by different levels of gene expression in the mammary gland (Salama et el., 2014 and unpublished results). For the milk fat, that HS caused a downregulation in the genes related to de novo fatty acids synthesis (acetyl-CoA carboxylase and fatty acid synthase), fatty acid desaturation (stearoyl-CoA desaturase), and milk fat globule formation (butyrophilin, subfamily-1-member A1, xanthine dehydrogenase, and glycosylation dependent cell adhesion

molecule 1). Moreover, s1-casein, s2-casein, -casein, lactotransferrin, and lactalbumin genes related to protein and lactose secretion were downregulated by HS. The reduced expression of lactoferrin gene could indicate that goats under HS might be more susceptible to mastitis as lactoferrin is an antimicrobial milk protein (Kutila et al., 2003). Moreover, there was gene upregulation for cathepsins (CTSB, CTSD, CTSS, and CTSZ) and plasminogen activator genes, which would indicate greater proteolysis in milk produced from HS goats and could explain the altered coagulation properties during cheese-making as it was explained previously. One of the most recognized cellular responses to hyperthermia is the increase in expression of heat shock proteins (HSP). Those HSP exert cytoprotective effects to assist folding of newly synthesized proteins and repairing and refolding damaged proteins under stress conditions (Kregel, 2002), avoiding cellular apoptosis. Bovine mammary epithelial cells incubated in vitro at high temperatures had increased HSP70 mRNA expression (Collier et al., 2008). Salama et al. (2014) also observed an increase in the expression of some HSP (e.g. HSPA5, HSPA9, and HSP90B1) in milk cells of HS dairy goats.

1.6 Strategies to reduce the impact of heat stress in dairy ruminants

1.6.1 Feeding manipulation

Heat increment for fermentation is higher for fiber than concentrates (Webster, 1983) and heat production is more associated with metabolism of acetate than with propionate. In small ruminants, heat production in the gut was not greatly affected by diet composition but increased exponentially with increasing metabolizable energy intake (Webster et al., 1975). Therefore, both the amount of intake and characteristics of fiber must be considered in designing an effective nutritional and environmental management program. Due to the reduction in feed intake, energy intake is a limiting factor in hot weather, and usually a common approach is increasing energy density, reducing forage, and increasing concentrate content of the ration. Generally, cattle under warm environments selectively decrease the quantity of forage consumed relative to concentrates (McDowell, 1972; Bernabucci et al., 1999). The increase of concentrates in a hot diet is a common practice. Coppock (1985) reported that maximal benefit from concentrates appears to be approximately 60 to 65% of the diet and an excessive concentrate feeding may lead to acidosis and the associated production, health, and metabolic difficulties.

In review papers, West (1999, 2003) reported that cows fed low fiber diets during hot weather had greater daily milk yield, lower body temperatures, and slower respiratory rates

compared with those fed high fiber diets. Moreover, the addition of fermentable carbohydrates has positive effects on DMI and milk energy produced per unit of feed energy. Probably, additional fermentable carbohydrates contribute to greater efficiency of energy use (West, 1999). However, considering the effects of heat exposure on rumen and intestine health, low fiber, high grain diets must be carefully balanced with the need for adequate fiber to promote chewing and rumination to maintain ruminal pH and cow health.

The addition of fat to the diet of lactating dairy ruminants is a common practice. The conversion of dietary fat to body fat is highly efficient when compared with the conversion of acetate to fatty acids (Baldwin et al., 1980). Moreover, feeding fat is associated with reduced metabolic heat production per unit of energy fed (Baldwin, et al., 1980) and compared to starch and fiber, fat has a much lower heat increment in the rumen (Van Soest, 1982). Therefore, feeding fats may improve efficiency and reduce heat increment and therefore may be beneficial during hot weather.

Results on the effects of fat supplementation under hot conditions are conflicting. Literature reports that feeding fat may be beneficial (O'Kelly, 1987; Skaar et al., 1989; Hamzaoui et al., 2013b) or ineffective (Bunting et al., 1992, 1996; Knapp and Grummer, 1991; White et al., 1992). Differences between studies carried out on ruminants are probably due to the fact that excess ruminally active fat in the diet may impair ruminal fermentation (Van Nevel and Demeyer, 1988). Only diets with 3 to 5% added fat have no toxic negative effects on ruminal microflora (Palmquist and Jenkins, 1980). However, ruminally protected fats allow the inclusion of a substantial quantity of fat in the diet, which could lower the heat increment significantly. Milk production and efficiency have been enhanced by feeding protected lipids (Kronfeld et al., 1980). Bernabucci, (2012) summarized some practical applications to add fat; not exceeding 5 to 7% total fat in the diet. Fat levels beyond these should be supplied using a rumen inert fat. As a general guideline, no more than 30 to 40% of total dietary fat should come from whole oil seeds (a source of unsaturated oils), 40 to 45% from other basal ingredients, and 15 to 30% from ruminally inert fats.

Due to the reduction of feed intake (and consequently N intake) N balance is decreased under HS (Ronchi et al., 1999; Shwartz et al., 2009; O'Brien et al., 2010; Hamzaoui et al., 2013a). Thus, increasing protein content of the hot diets above requirements seems advantageous, but it should be considered that feeding excess protein is associated with an increase in energy cost. Tyrrell et al. (1970) reported that dietary N above requirement reduces metabolizable energy by 7.2 kcal/g of N. The energy cost associated with synthesizing and excreting urea accounted for the reduced milk yield (Danfaer et al., 1980;

Oldham, 1984). This is corroborated by the fact that the energy necessary to form urea from excess protein appears as heat production and decreases the proportion of net energy for lactation. In this regard, Ames and Brink (1977) and Hassan and Roussel (1975) concluded that it seems logical that altering protein to match thermal environments would improve protein efficiency ratio of growing animals.

Other than the amount of proteins fed, quality of protein sources (solubility and/or degradability, and biological value) should be taken into account, especially under HS conditions. Studies investigating dietary protein content as well as composition suggest an interaction between protein availability and environment, and indicate that dietary protein degradability may be particularly critical under HS conditions (Zook, 1982; White et al., 1992). Huber et al. (1994) suggested that when cows are subject to hot weather conditions ruminal degradable proteins should not exceed 61% of dietary CP, and total protein should not exceed recommendations by greater than 100 g N/d. Thus, during HS, it is necessary to increase the protein level of the ration, but this increase should be provided as rumen undegradable protein or improving protein quality and essential amino acids (lysine in particular).

Cows subjected to hot climatic conditions may experience acid-base disturbances resulting from respiratory alkalosis (due to panting; Schneider et al., 1984), with subsequent renal compensation by increasing urinary excretion of bicarbonate and Na, renal conservation of K (Collier et al., 1982), and increase of Cl retention (Escobosa et al., 1984; West et al., 1991; Ronchi et al., 1995). These responses are responsible for changes in the blood cation-anion balance (CAB = Na+K – Cl as described by West et al., 1992). Since electrolytes are a key element in acid-base chemistry their supplementation may be critical to homeostatic mechanisms during HS. In this regard, studies carried out on heat-stressed lactating cows (Mallonee et al., 1985; Schneider et al., 1984; West et al., 1987) reported positive effects of K supplementation above minimum recommendations (NRC, 1989) on milk yield. Moreover, Schneider et al. (1986) supplemented cows with 0.55% Na during hot climatic conditions and found greater feed intake and milk yield compared with cows that received 0.18% Na. Similarly, Silanikove et al. (1998) observed higher DMI, milk, protein, fat, and lactose yields in cows receiving a ration with increased amounts of Na, K, and Cl, compared with cows that consumed the same ration with a lower concentration of these ions.

West et al. (1992) reported that during HS conditions DMI was improved as dietary cation-anion difference (DCAD) was increased from 120 to 464 mEq Na+K− Cl/100 g feed DM. The improved intake and/or milk yield were observed when more alkaline diets were fed to

lactating cows, and this may result from improved blood buffering or correction of mineral deficiencies. The need for alkaline diets is consistent with addition of buffers to the diet, since the ideal means to increase DCAB for lactating cows is with Na or K in association with a metabolizable ion such as bicarbonate.

Stress (including HS) increases vitamin mobilization from tissues and their excretion (Siegel 1995), and thus may exacerbate a marginal vitamin deficiency or an increased vitamin requirement. The HS generally increases the production of free radicals, leading to oxidative stress (Bernabucci et al., 2002; Pamok et al., 2009; Azad et al., 2010). Vit-E and Vit-C supplementation may mitigate the negative effects of HS by reducing the oxidative stress.

Cattle supplemented with niacin (6 g/d) during summer increased milk yield by 0.9 kg/d compared with controls (Muller et al., 1986). Those authors speculated that niacin improved milk yield by affecting lipid and energy metabolism, by stimulating protein synthesis by ruminal microorganisms, or by causing other effects on ruminal microorganisms. On the other hand, Di Costanzo et al. (1997) reported that supplementation with niacin (12, 24, or 36 g/cow•d $^{-1}$) resulted in a reduction of skin temperatures of about 0.3ºC, but rectal temperature and milk yield were unchanged.

Water is undoubtedly the most important nutrient for animals subjected to HS for cooling by evaporation. The heat required to convert water into vapor is referred to as the latent heat of vaporization. The vaporization of 1 ml of water requires 2.43 J and this is the amount of heat lost when 1 ml of sweat evaporates from the skin. Berman et al. (1985) reported that the maximal rate of water evaporation in lactating cows was 1.5 kg/h, which is equivalent to 4.3 kJ/d.

Water requirements have not been extensively investigated probably because water is usually supplied in abundance. Many studies show significant positive correlations between water intake and ambient temperature (NRC, 1989). It is obvious that abundant water must be available at all times under hot conditions. In climate-controlled chambers water intake of lactating cows increased 29% at the warmer temperature, fecal water loss declined 33%, but loss of water via urine, skin surface, and respiratory evaporation increased 15, 59, and 50%, respectively (McDowell, 1972). Consumed water has a direct cooling effect via the reticulo-rumen (Bianca, 1964). Cows offered drinking water at 10ºC or 28 to 30.2ºC consumed more DM (Baker et al., 1988; Milam et al., 1986) and yielded more milk in the cool water treatment (Milam et al., 1986).

1.6.2 Environmental modifications

In modern dairy facilities dairy producers strive to achieve consistently high milk production, feed efficiency, and reproductive efficiency while maintaining the health of the dairy animals. The use of low-pressure sprinklers, soakers, and fan systems to effectively wet and dry cows will increase heat loss from the cow. Dairy cows can be soaked in the holding pen, exit lanes, and at the feed lines. The goal should be to maximize the number of wet and dry cycles per hour. Brouk et al. (2002) conducted an 8×8 replicated Latin Square experiment in which 8 cows were exposed to each of eight different cooling strategies. The THI during the study was 80. Each cow was exposed to treatments involving no cooling, fan-only cooling, soaking 1 minute out of every 5 minutes, soaking 1 minute out of every 10 minutes, soaking 1 minute out of every 15 minutes, or a combination of a fan with each of the soaking strategies. They concluded that different cooling strategies could be developed for different levels of HS. Collier et al. (2006) reviewed the major advances associated with environmental effects on dairy cattle. They discussed widely the impact of cooling systems and facilities on HS in dairy cows. Later, Smith and Harner (2012) also reviewed the different strategies to reduce the impact of HS in dairy cattle facilities. Listed below are the priorities for reducing HS in dairy facilities:

1. Improve water availability
2. Provide shade in the housing areas and holding pen
3. Reduce walking distance
4. Reduce time in the holding pen
5. Improve holding pen ventilation
6. Add holding pen cooling and exit lane cooling
7. Improve ventilation in cow housing areas (freestalls)
8. Cool close-up cows (3 weeks prior to calving)
9. Cool fresh and lactating cows

Various cooling systems have been evaluated rather than the aforementioned systems, but the cost is the limitating factor for the producers. Amongs them, the air conditioning, Zone-cooled cows (cooled air blown over the head and neck), Low-Profile Cross-Ventilated Freestall Buildings (LPCV) etc. The costs associated with those systems, and facilities necessary to provide an enclosed environment, or ducting for zone cooling have proven cost-prohibitive, and these types of systems are rare today.

1.6.3 Genetic selection

The impact of HS can be relieved by the aforementioned modification of the feeding, environment or by genetic selection of animals less affected by thermal stress. Identification of such animals can be based on measurements of their immediate response to the exposure to HS conditions. Selection for heat tolerance in ruminants could be very expensive due to a long generation interval. If heat tolerance in dairy ruminants is decreasing as a correlated response of selection for production, dairy ruminants may no longer be profitable in hot climates. Higher heat tolerance can be achieved by selecting or crossbreeding with more heat-tolerant animals. Crossbreds in dairy cattle have been successful under extensive, but not intensive, management because of lower production levels than purebreds (McDowell et al., 1996). Also, while the F1 may be heat-tolerant, the more complex crossbreds may be less (Rutledge, 2001). Therefore, the remaining option is to select for more heat-resistant purebreds. Misztal and Lovendahl (2012) proposed several conditions needed for the selection to be successful:

1) The genetic component of HS should be high enough to create a potential for improvement.

2) Heat tolerant animals must be identified with sufficient accuracy.

3) The environmental modifications must be unable or too expensive to address the problem of heat tolerance in the long run, in comparison to genetic selection.

Ravagnolo and Misztal (2000) suggested that the major obstacle for selection is the availability of data for such selection on national basis. While the THI is widely used as indicator of external heat load caused by a combination of temperature and relative humidity, those authors presented a methodology for genetic evaluation for heat tolerance based on data from weather stations. When that method was applied for milk yield of Holsteins in Georgia, the genetic correlation between a traditionally predicted transmitting ability and predicted transmitting ability for heat tolerance was about -0.4, and the variance of heat tolerance predicted transmitting ability was large at high THI. Thus quantitative genetic analyses of Ravagnolo and Misztal (2000) revealed a high genetic variability of milk production in Holsteins at extreme temperature and humidity, indicating the possibility for selection for heat tolerance.

Later, Bohmanova et al. (2005) developed a national genetic evaluation for HS in United States. Hourly temperature and relative humidity records were available from 202 public

weather stations across the United States. Herds were assigned by distance to the nearest weather station. Sires that were most heat tolerant transmitted lower milk yields with higher fat and protein contents than did sires that were least heat tolerant. Daughters of the most heat-tolerant sires had better type, worse dairy form, better udder and body composites, higher Type-Production Indexes, longer productive life, and higher daughter pregnancy rates than did daughters of the least heat-tolerant sires. Important questions are whether public weather stations provide accurate information and whether more genetic variance may be captured with on-farm measurement of THI.

Another option to get heat tolerant animals is the creation of one or more lines. Creation of a line could be very expensive in cattle due to a long generation interval; although, the use of genomic selection can reduce costs and the generation interval dramatically. Front the little evidence that is available on genes underlying heat tolerance, heat shock genes have been widely discussed as candidate genes for heat resistance (Hoffmann et al., 2003). Although the phenomenon of cross resistance where exposure to one stressor enhances resistance to other stressors has been noted by Hoffmann et al. (2003). This suggests that HS tolerant cattle may be also tolerant to other stressors such as disease, reduced feed quality, parasites. Such stress tolerant cows may have lower culling rates and thus may stay in herds longer. This fact has been confirmed in Drosophila, where a relationship between heat resistance and longevity has been found. However, whether this is valid in ruminants is unknown (Sørensen et al., 2003).

CHAPTER 2

Objectives

CHAPTER 2

OBJETIVES

2.1 General objectives

Dairy goat farming is of relevant economic importance in the Mediterranean area, where developed countries such as Greece, Italy, France, and Spain have an important dairy goat industry, with an increasing demand for goat's milk and its products. However, farm animals in many areas of the aforementioned countries, are exposed annually to 3–5 months of considerable heat stress. Moreover, the severity and frequency of heat waves are expected to increase throughout the next years. Many studies have been published on the response of dairy cows to heat stress, but little is known about the performance of heat-stressed dairy goats.

Keeping this fact in mind, the general objective of the current thesis was on one hand to evaluate different types of response to heat stress (Chapters 3 and 4), and on the other hand test the effectivity of some nutritional strategies to mitigate its effects (Chapters 5 and 6).

Keeping this fact in mind, the general objective of the current thesis was on one hand to evaluate different types of response to heat stress (Chapters 3 and 4), and on the other hand test the effectivity of some nutritional strategies to mitigate its effects (Chapters 5 and 6).

2.2 Specific objectives

2.2.1 Responses of dairy goats to heat stress:

Goats were exposed to heat stress and the following items were evaluated:

- **Physiological:**
  - Rectal temperature
  - Respiration rate
  - Insulin and epinephrine challenges
  - Glucose tolerance test
- **Lactational:**
  - Milk yield and composition at different stages of lactation
  - Milk fatty acid profile
- **Nutritional:**

- Dry mater intake
- Water consumption and water balance
- Digestibility (DM, OM, CP, NDF and ADF)
- Nitrogen balance

- **Metabolites in blood:**
  - Non esterified fatty acids
  - β-Hydroxybutyrate
  - Glucose and insulin
- **Acid-base indicators in blood:**
  - Sodium, potassium, and chloride
  - Bicarbonate, total CO_2, pressure of CO_2, anion gap, and base excess
  - Hematocrit and haemoglobin
- **Stress indicators:**
  - Haptoglobin concentration in blood
  - Fecal Corticosterone
- **Behavior changes throughout the day (daylight and night)**
  - Feeding and drinking bouts and durations
  - Frequency of changing the position (standing and lying down)
  - Time budget of different activities throughout the day

2.2.2. Nutritional manipulation to alleviate the negative effects of heat stress:

Based on the results obtained in the first section, the ration of dairy goats was supplemented with:

- **Soybean oil:** as a source of fatty acids to improve the quantity and quality of goat milk produced under heat stress conditions
- **Propylene glycol:** as a glucogenic component to increase blood glucose and spare more amino acids for milk protein synthesis.

CHAPTER 3

Physiological responses and lactational performances of late lactating dairy goats under heat stress conditions

CHAPTER 3

Physiological responses and lactational performances of late lactating dairy goats under heat stress conditions

3.1 ABSTRACT

Eight Murciano-Granadina dairy goats in late lactation were exposed to different ambient conditions, using metabolic cages in a climatic chamber. Experimental design was a crossover (2 periods of 35 d and 4 goats each), and conditions were: 1) thermal neutral (TN, 15 to 20°C day-night), and 2) heat stress (HS, 12-h day at 37°C and 12-h night at 30.5°C). Humidity was maintained at 40% and light-dark was constant (12-12 h). Forage:concentrate ratio was adjusted daily for maintaining similar value in TN and HS goats (70:30). Water was freely available at ambient temperature. Rectal temperature and respiratory rate (0800, 1200 and 1700 h) and milk yield were recorded daily, whereas milk composition, nonesterified fatty acids (NEFA) and haptoglobin in blood were analyzed weekly. At d 25, additional blood samples were taken for analysis of metabolites and indicators of the acid-base balance. Digestibility coefficients and N balance were determined (d 31 to 35) and body weight was recorded (d 35). Compared to TN goats, HS goats experienced greater rectal temperature (+0.58 ºC), respiratory rate (+48 breaths/min), water intake (+77%) and water evaporation (+207%). Intake of HS goats rapidly declined until d 7 (–40%), partially recovered from d 7 to 19, and steadied thereafter (–14%). No changes in digestibility or N balance were detected. Blood NEFA and haptoglobin peaked at d 7 in HS goats but did not vary thereafter. Although milk yield did not vary by treatment, milk of HS goats contained –12.5% protein and –11.5% casein than TN goats. Panting reduced concentration and pressure of CO_2 in blood of HS goats, but they were able to maintain their blood pH similar to TN group by lowering HCO_3^- and increasing Cl^- concentrations in blood. In conclusion, heat-stressed dairy goats showed dramatic physiological changes during the first week of treatment and partially recovered thereafter. They were able to maintain milk yield by losing body mass, but milk protein content and protein yield were depressed. Further research is needed to assess the response of dairy goats to heat stress at earlier stages of lactation.

3.2 INTRODUCTION

Heat stress (HS) decreases milk production of dairy animals, and a half of this reduction in milk yield is due to reduced DMI (Rhoads et al., 2009). The other half of milk yield losses could be explained by the increase in maintenance requirements (NRC, 2007), decreasing secretion of growth hormone (Mitra et al., 1972), lowering blood flow to the udder (Lough et al., 1990), down-regulating milk protein genes and up-regulating apoptosis genes in the mammary gland (Collier et al., 2006). Cows under HS had greater levels of insulin with improved insulin sensitivity and lacked the ability of fat mobilization from adipose tissue to face the decreased DMI (Baumgard and Rhoads, 2013). The reduction in dairy farm profit associated with HS when the temperature-humidity index (THI) is extremely high is not only a result of decreased milk yield, but also includes impaired milk quality, reproduction problems, increased health care costs, and even animal death.

Despite the large number of studies carried out in dairy cows, little is known about the effects of HS in dairy goats. Goats are considered more tolerant to high THI values compared to dairy cows because of their metabolic size and high water conservation capacity (Silanikove, 2000). When environmental temperatures increased from 20 to 40°C, respiration rate increased from 30 to over 200 breaths/min in East African goats (Maloiy and Taylor, 1971) and domestic Swedish goats (Olsson et al., 1995), indicating that water evaporation by respiration plays an important role in heat dissipation in goats.

Lactating Saanen goats exposed to moderate or severe HS for 4 d (THI, 81 or 89) lost milk yield by 3 or 13%, respectively (Sano et al., 1985). Brown et al. (1988) reported that the exposure of dairy goats to moderate HS conditions for 5 wk (34ºC and 25% humidity; THI = 79) depressed milk yield in Alpine but not in Nubian goats, indicating that the response to HS varies according to breed.

The objective of the current study was to measure the physiological, lactational, and nutritional responses to extreme heat stress conditions in Spanish Murciano-Granadina dairy goats at late lactation. Moreover, blood acid-base status and stress indicators were also evaluated. No information is available on the effects of heat stress on this dairy breed, which is widely spread in the Mediterranean area.

3.3 MATERIALS AND METHODS

3.3.1 Animals and Management Conditions

Animal care conditions and management practices agreed with the procedures stated by the Ethical Committee of Animal and Human Experimentation of the Universitat Autonoma de Barcelona (CEEAH reference 09/771) and the codes of recommendations for the welfare of livestock of the Ministry of Agriculture, Food and Environment of Spain.

Eight open multiparous Murciano-Granadina dairy goats (43.5 ± 2.6 kg BW) with healthy and symmetrical udders, from the herd of the experimental farm of the Universitat Autònoma de Barcelona were blocked in 2 balanced groups and used at late lactation (194 ± 3 DIM; 1.53 ± 0.04 L/d).

The experimental design was a crossover with 2 treatments in 2 periods, lasting 35 d, and 4 goats each. Goats were switched to the opposite treatment in the second period. Climatic conditions were: 1) thermal neutral (TN; 15 to 20°C and 45%; THI = 59 to 65), and 2) heat stress (HS; 12-h day at 37°C and 40%; THI = 85; and 12-h night at 30.5°C and 40%; THI = 77). Order of treatments on each goat was recorded and taken into account in the statistical analyses. The THI values were calculated according to NRC (1971) as follows:

$THI = (1.8 \times T_{db} + 32) - [(0.55 - 0.0055 \times RH) \times (1.8 \times T_{db} - 26.8)]$, where T_{db} is the dry bulb temperature (ºC) and RH is the relative humidity (%).

Throughout the experiment, (mid-January to mid-April), the TN goats were kept indoors and the temperature was maintained at 15 to 20ºC with the help of electric heather equipped with a thermostat (3.5 kW; General Electric, Barcelona, Spain). Temperature and relative humidity averaged 16.7 ± 0.3ºC and 45 ± 5% (THI = 61) for the TN goats. The HS goats were kept in a 4×6×2.3 m climatic chamber (Euroshield, ETS Lindgren-Euroshield Oy, Eura, Finland) provided with a temperature and humidity controlling system (CAREL Controls Ibérica, S.L., Barcelona, Spain). A continuous 90 m^3/h air turnover was maintained throughout the experiment.

Goats had a 4-wk pre-experimental period under TN conditions for the adaptation to the diet and to metabolic cages. When goats were switched from TN to HS conditions, a transition period of 2 d was allowed (1 d at 25°C and 1 d at 30°C), but no transition was applied for the change from HS to TN. Photoperiod was maintained constant at 12-12 h light-dark (0900 to 2100 h) and data of environmental temperature and humidity were recorded every 10 min by using 2 data loggers (Opus 10, Lufft, Fellbach, Germany).

Daily ration of the goats consisted of (as fed) dehydrated fescue hay ad libitum (20% daily refusal), 0.65 kg alfalfa pellets, and 0.8 kg of concentrate mixture (corn, 30%; barley, 25.8%; soybean meal, 25%; sunflower meal, 8.5%; fatty acid sodium salts, 5%; dicalcium phosphate, 2.5%; calcium carbonate, 2%; sodium chloride, 1%; and vitamins A, E, and D3; as fed). Mineralized salt blocks were freely available in each metabolic cage (Composition: Na, 36.74%; Ca, 0.32; Mg, 1.09%; Zn, 5 g/kg; Mn, 1.5 g/kg; S, 912 mg/kg; Fe, 304 mg/kg; I, 75 mg/kg; Co, 50 mg/kg; Se, 25 mg/kg; Ovi bloc, Sal Cupido, Terrasa, Spain). The concentrate mixture was offered in 2 daily portions at 0900 and 1600 h. Changes in the forage intake of the HS goats were taken into account throughout the experiment and the amount of concentrate offered was daily modified to maintain a constant and similar forage:concentrate ratio to that of the TN goats. Clean water was permanently available at ambient temperature, according to treatment.

Goats were milked once daily (0800 h) with a portable milking machine (Westfalia-Separator Ibérica, Granollers, Spain) set at 42 kPa, 90 pulses/min, and 66% pulsation ratio, provided of recording jars (2 L ± 5%). Milking routine included cluster attachment without udder preparation or teat cleaning, machine milking, machine stripping before cluster removal, and teat dipping in an iodine solution (P3-ioshield, Ecolab Hispano-Portuguesa, Barcelona, Spain).

3.3.2 Sample Collection, Analyses, and Measurements

3.3.2.1 Body Temperature and Respiration Rate

Rectal temperatures and respiration rates were recorded at 0800, 1200, and 1700 h. Rectal temperature was measured by a digital clinical thermometer (Model ICO Technology "mini color", Barcelona, Spain; range, 32 to 43.9ºC; accuracy, ± 0.1ºC), whereas number of inhalations and exhalations during 60 s indicated the respiration rate.

3.3.2.2 Feed Intake and Water Consumption

Feed intake and water consumption (accuracy, ± 20 g) were recorded daily throughout the experiment. Trays with saw dust were put below the drinking troughs and weighted twice daily to take into account water wastes. Feed samples were collected before the beginning of each experimental period and were ground through a 1 mm stainless steel screen, and then analyzed for DM, ADF, NDF, and ash according to analytical standard methods (AOAC, 2003). The Dumas method (AOAC International, 2003) with a Leco analyzer (Leco

Corporation, St. Joseph, MI) was used for N determinations and CP was calculated as percentage of N×6.25. The chemical composition and nutritive value of ration ingredients are shown in Table 3.1.

Table 3.1. Chemical composition and nutritive value (DM basis) of the ration ingredients used for dairy goats

Item	Fescue hay	Alfalfa pellets	Concentrate
Component, %			
DM	89.99	93.04	90.30
OM	89.70	86.50	88.60
CP	10.60	12.60	17.90
NDF	48.10	44.90	12.60
ADF	23.30	27.00	6.19
Nutritive value[1]			
UEm/kg[2]	1.57	-	-
UFL/kg[3]	0.57	0.68	1.17
NE_L, Mcal/kg	0.97	1.16	1.99
PDIE[4], g/kg	66	83	129
PDIN[5], g/kg	57	95	153
PDIA[6], g/kg	24	47	79
Ca, g/kg	3.5	16.5	18.8
P, g/kg	2.5	2.5	9.1

[1]Calculated according to INRA (2007).[2]Fill units for sheep (1 UEm = 1 kg DM reference grass).[3]Feed units for lactation (1 UFL = 1.7 Mcal EN_L).[4]Protein digested in the small intestine supplied by microbial protein from rumen-fermented organic matter.[5]Protein digested in the small Intestine supplied by microbial protein from rumen-degraded protein.[6]Protein digested in the small Intestine supplied by rumen-undegraded dietary protein.

3.3.2.3 Milk Yield and Milk Composition

Milk yield of individual goats was recorded daily throughout the experiment and milk composition was evaluated weekly. A milk sample of approximately 100 mL was collected and preserved with an antimicrobial tablet (Bronopol, Broad Spectrum Microtabs II, D&F Control Systems Inc., San Ramon, CA) at 4°C until analysis. Milk samples were analyzed with a near-infrared spectrometer (Foss NIRSystems 5000, Foss, Hillerød, Denmark) for contents of TS, fat, total protein (N × 6.38), true protein, and CN. Whey protein was calculated by the difference between true protein and CN, and NPN was calculated by the difference between total protein and true protein.

3.3.2.4 Blood Measures

Blood samples were taken weekly from the jugular vein into 10 mL plastic lavender-vacutainers with spray-coated K2-EDTA (BD Diagnostics, Franklin Lakes, NJ) before the morning feeding. Plasma was obtained by centrifugation of whole blood for 15 min at 1500 × g, and stored at –20°C for the NEFA and haptoglobin analyses. The NEFA were determined by the colorimetric enzymatic test ACS-ACOD method using a commercial kit (Wako Chemicals, Neuss, Germany). Haptoglobin was determined colorimetrically by the hemoglobin binding method using a commercial haptoglobin assay (Assay Phase Range, Tridelta Development, Maynooth, Ireland) and an Olympus AU400 analyzer (Olympus Europa, Hamburg, Germany).

At d 25, blood samples (approximately 0.3 mL) were collected by insulin syringes (1 mL; BD Micro-Fine, BD Medical-Diabetes Care, Franklin Lakes, NJ) at 0800 and at 1700 h and immediately analyzed for major ions and metabolites. A single drop of blood was applied to disposable cartridges containing biochemical and silicon chip technology (i-STAT EC8+, Abbott Point of Care, Princeton, NJ). Then, the cartridge was inserted into an i-STAT handheld analyzer, and the results of glucose, urea, Cl, Na, K, total CO_2 concentration, anion gap, hematocrit, hemoglobin, pH, partial pressure of CO_2, HCO_3^-, and base excess were obtained.

3.3.2.5 Digestibility Coefficients, and Water and N Balances

Feed orts were daily collected (d 31 to 35), weighed, and composted for analysis. Feces of each goat were daily collected and 10% of fresh feces were dried at 60°C for 48 h. Then a composted sample for each goat was stored at room temperature until analysis. Urine was collected in containers with 20 mL of H_2SO_4 (96%) and urine volume was daily measured (accuracy, ± 2 mL). Urine samples (5% of total volume) were composted and stored at –25°C for N content analysis. Samples of urine without H_2SO_4 were collected at 0800 and at 1700 h during the last 2 experimental days to measure urine pH. Orts and feces samples were ground through a 1 mm stainless steel screen and then analyzed for DM, CP, CF, ADF, NDF and ash, as previously indicated. Water balance was also done during the digestibility period.

3.3.2.6 Corticosterone in Feces

Samples of fresh feces were collected on the last day of each experimental period and stored at –25ºC for corticosterone analysis. Fecal samples were first lyophilized, then were

extracted with methanol, and finally diluted 1:10 with the assay buffer of the kit. Analyses were performed in the Clinical Biochemistry Service of the Veterinary Faculty of the Universitat Autònoma de Barcelona (Bellaterra, Barcelona, Spain) using the commercially available 125I RIA kit (Rats and Mice Corticosterone kit, ICN Pharmaceuticals, Orangeburg, NY), as described by Morrow et al. (2002). Recovery of known amounts of corticosterone added to the processed samples was 59.8%. The relationship between theoretical and true values of corticosterone in goat feces was linear ($y = 0.9919x – 2.863$; $r^2 = 0.997$). The inter- and intra-assay coefficients of variation were 16.8 and 9.5%, respectively.

3.3.3 Statistical Analyses

Data were analyzed by the PROC MIXED for repeated measurements of SAS version 9.1.3 (SAS Inst. Inc., Cary, NC). The statistical mixed model contained the fixed effects of the treatment (HS vs. TN), day and period; the random effect of the animal; the interactions treatment × day and treatment × period; and the residual error. The model took into account the possible carryover effects of previous HS periods through the treatment × period interaction. Data of performances (i.e., intake, water, milk yield) and physiological indicators (i.e., rectal temperature and respiratory rate) were analyzed on daily basis.

For blood parameters measured at 0800 and 1700, the model included the effects of treatment, sampling hour and period; and the interaction treatment × period and treatment × hour. Data on digestibility and nutrient balance were analyzed using PROC GLM of SAS. The model contained the effect of treatment and period; the interaction treatment × period; and the residual error.

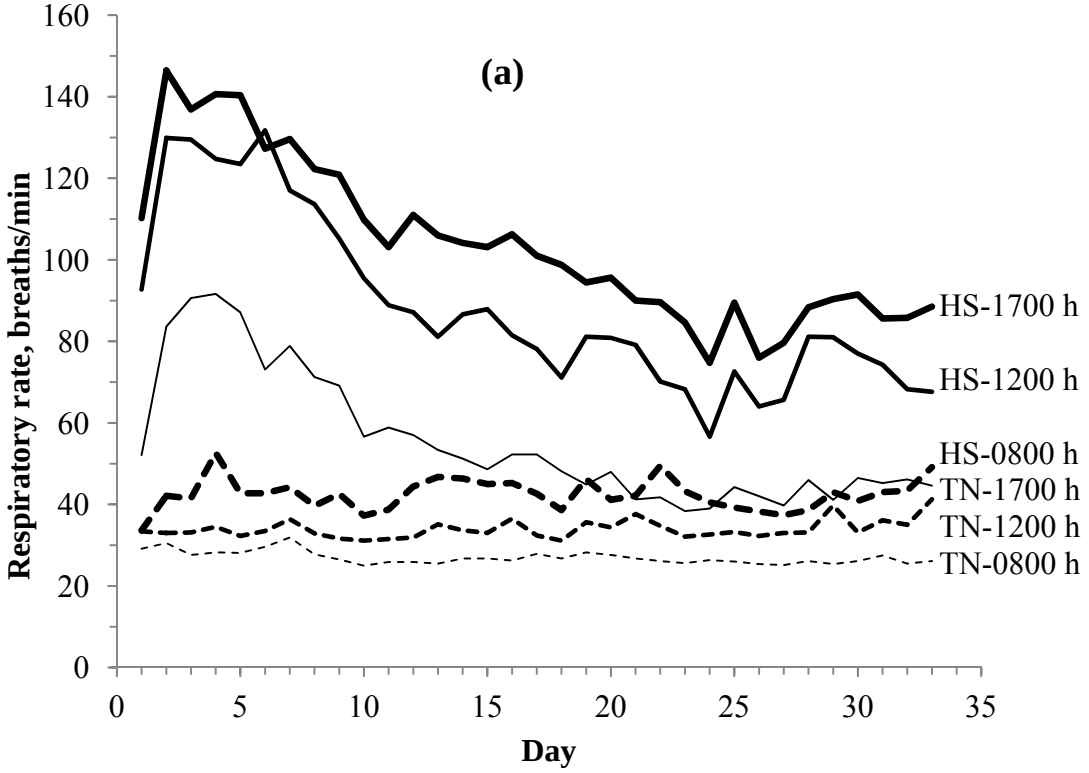
Data were tested for the normality of distribution, and a logarithmic transformation (log10) was applied to haptoglobin concentration in blood. Differences between least squares means were determined with the PDIFF test of SAS. Significance was declared at $P< 0.05$ unless otherwise indicated.

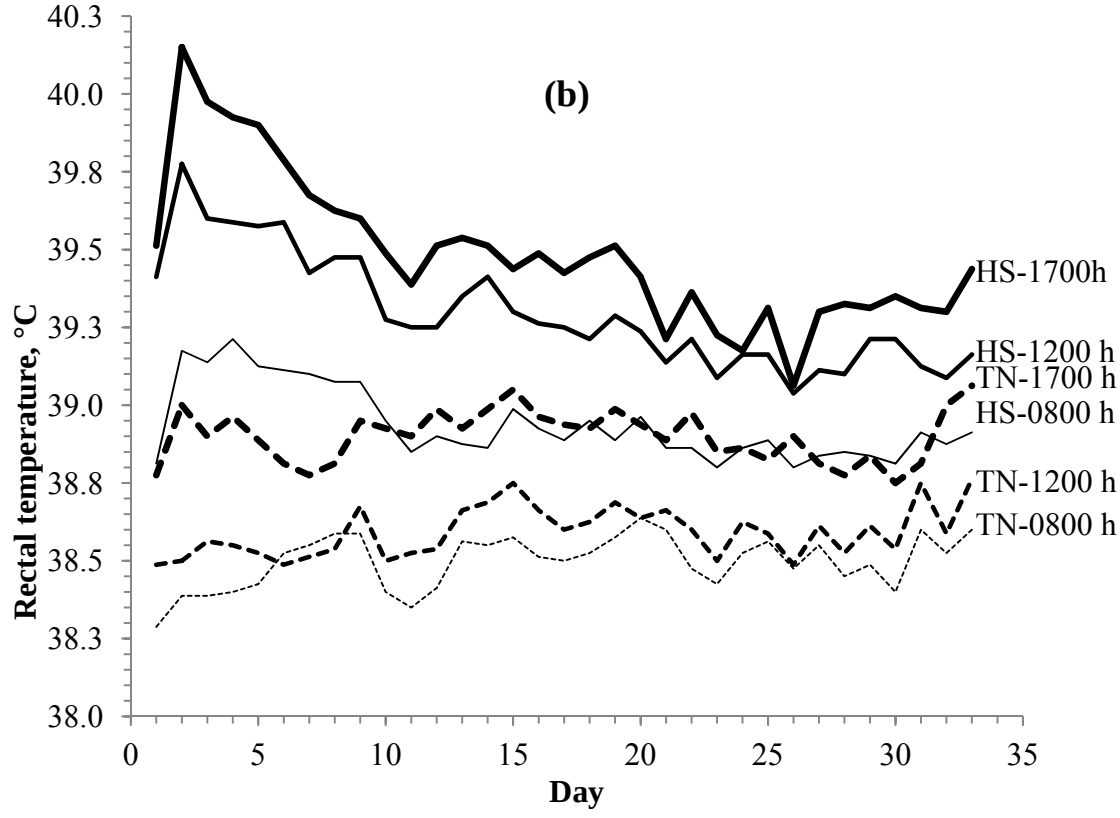
3.4 RESULTS AND DISCUSSION

3.4.1 Rectal Temperature and Respiration Rate

Rectal temperatures and respiration rates increased from 0800 to 1700 h in both goat groups, but were greater in HS than in TN goats at all-time points (Figure 3.1a and 4.1b; $P< 0.001$).

Figure 3.1. Rectal temperature (a) and respiratory rate (b) at different hours during the day (0800, 1200, and 1700 h) in dairy goats under thermal neutral (TN, dashed lines, n = 8) or heat stress (HS, solid lines, n = 8) conditions at late lactation.





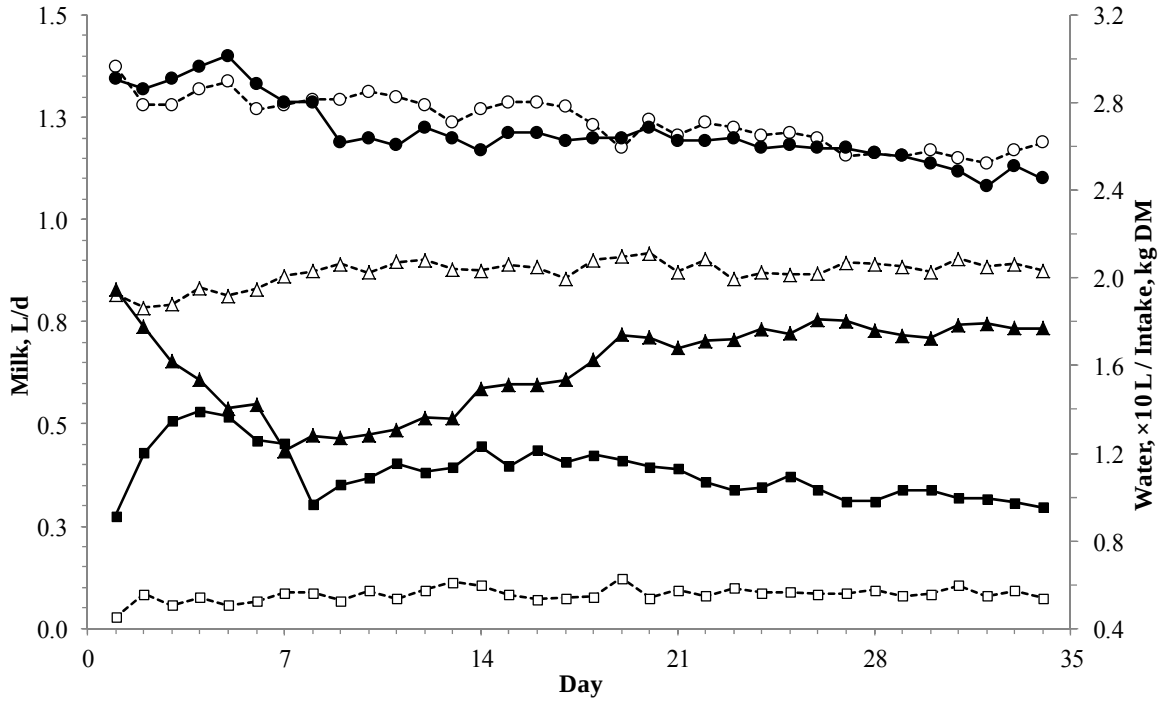
The increased rectal temperatures and respiration rates in TN goats from 0800 to 1700 h were in accordance with the increment of ambient temperature throughout the day (15 to 20ºC). Comparing the HS goats at 0800 h (after being exposed to 30.5ºC during the night; THI = 77) with TN goats at 1700 h (THI = 65), we observed similar rectal temperatures (+0.04ºC; $P = 0.390$) but greater respiration rates (+13 breaths/min; $P< 0.01$), indicating that HS goats were under heat stress throughout the day, but to a lower extent during the night. Reference respiratory rate for adult goats ranges between 15 and 30 breaths/min according to Pugh and Baird (2012), but it was greater in our TN goats at 1700 h probably because of breed and physiological state differences. Maximum rectal temperature difference (+0.70ºC; $P< 0.001$) between HS and TN goats occurred at 1200 h, whereas the largest difference in respiration rate (+65 breaths/min; $P< 0.001$) occurred at 1700 h. Increased respiration rate under HS conditions is a known mechanism for dissipating heat load by evaporation. Rectal temperature and respiration rate values peaked in HS goats during the first week and then gradually decreased, which indicates a partial adaptation to the HS conditions.

3.4.2 Feed Intake

On average, DMI decreased by 21% throughout the 35-d experimental period but showed a marked effect of time elapsed after the start of the HS treatment (Figure 3.2; $P< 0.001$).Feed intake under HS conditions gradually decreased during wk 1, partially recovered during wk 2 and 3, and remained constant thereafter (Figure 3.2). Heat stress caused a 27% reduction in DMI from d 1 to 19 (1.47 ± 0.05 vs. 2.00 ± 0.06 kg/d; $P < 0.001$) and 14% from d 20 to 35 (1.75 ± 0.06 vs. 2.03 ± 0.06 kg/d; $P < 0.001$). The partial recovery of DMI from d 19 onwards indicates the adaptation of goats to HS conditions. Previous studies comparing TN and HS under chamber controlled conditions in dairy cows (Rhoads et al., 2009; Shwartz et al., 2009) did not show such an adaptation.

Feed intake reduction due to HS has been previously reported in dairy goats (Sano et al., 1985), ewes (Abdalla et al., 1993), and cows (Lough et al., 1990; Rhoads et al., 2009). Heat-stressed animals decreased feed intake in an attempt to create less metabolic heat because the heat increment of feeding, especially in ruminants, is an important source of heat production (Kadzere et al., 2002). Moreover, the gut fill by water observed in the current study for HS goats (see later) might also be related to the reduced DMI.

Figure 3.2. Milk yield (○, ●), dry matter intake (Δ, ▲), and water consumption (□, ■) of dairy goats under thermal neutral (○, Δ, □ with dashed lines; n = 8) or heat stress (●, ▲, ■ with solid lines; n = 8) conditions at late lactation. The SEM values of milk yield, DM intake, and water consumption are 0.05 L, 0.06 kg, and 1.14 L, respectively.



According to the lactational performances of our goats (Table 3.2) and the requirements estimated according to INRA (2007), TN goats showed a greater feed intake (2.03 kg DM/d or 4.6% of BW) than predicted (1.72 kg DM/d or 3.9% of BW), which allowed them to cover their daily requirements (1.16 UFL and 102 g PDI) and to have positive energy and protein balances (+0.29 Mcal EN_L and +80 g PDI). Moreover, TN goats gained 1.8 kg (+51 g/d) during the experiment as it can be calculated from data shown in Table 2. On the other hand, feed intake of HS goats was lower (1.60 kg DM/d or 3.7% of BW) than predicted (1.72 kg DM/d or 3.9% of BW). If we also take into account a 30% increase in maintenance requirements because of HS as indicated by NRC (2007), the energy intake would not be enough to cover the daily requirements and resulted in an apparent BW loss of 1.5 kg (–41 g/d). The apparent BW changes in TN and HS goats included the inevitable variations in the digestive tract content, which were unknown in our data.

3.4.3 Water Consumption and Water Balance

Results of water consumption throughout the experiment and water balance measured from d 30 to 35 are shown in Tables 4.2 and 4.3, respectively.

Table 3.2. Lactational performances of Murciano-Granadina dairy goats under thermal neutral (TN, n = 8) and heat stress (HS, n = 8) conditions at late lactation (values are least squares means and SE of the difference).

Item	Treatment		SED	Effect (*P*<)		
	TN	HS		Treatment	Period	T × P[3]
Initial BW, Kg	43.6	44.1	1.3	0.720	0.576	0.832
Final BW, Kg	45.4	42.6	2.6	0.034	0.687	0.047
DMI, kg/d	2.03	1.60	0.08	0.001	0.010	0.152
Water consumption, L/d	5.5	11.1	1.20	0.001	0.322	0.468
Milk yield, L/d	1.24	1.21	0.02	0.198	0.001	0.674
FCM 3.5%, L/d[1]	1.38	1.35	0.07	0.529	0.004	0.226
Milk composition, %						
Total solids	12.89	12.41	0.29	0.259	0.625	0.734
Fat	4.21	4.22	0.19	0.961	0.898	0.124
Protein	3.84	3.36	0.15	0.030	0.793	0.986
True protein	3.62	3.12	0.14	0.022	0.977	0.965
Casein	3.21	2.84	0.12	0.034	0.179	0.709
Casein, % protein	84.1	84.9	0.64	0.200	0.001	0.091
Whey protein	0.63	0.53	0.04	0.029	0.001	0.332
NPN	0.22	0.24	0.01	0.155	0.001	0.797
Fat yield, g/d	52	51	2.9	0.678	0.021	0.210
Protein yield, g/d	48	40	1.8	0.001	0.220	0.183
NEFA, mmol/L plasma[2]	0.192	0.137	0.031	0.081	0.673	0.335

[1]Fat corrected milk at 3.5%; FCM = L × [0.432 + 0.162 × (fat %)], being L liters of milk yield.[2]Average values of blood samples collected weekly from d 0 to d 35.[3]Interaction Treatment × Period.

The HS goats had greater water consumption compared to TN goats (+5.50 L/d; *P*< 0.001). The greatest values of water intake were recorded during wk 1 when DMI was at its lowest value. However, water intake values stabilized earlier than DMI in the HS goats (Figure 3.2) and remained greater ($P < 0.01$) than in TN goats throughout the experiment. Increased water intake was mainly used by HS goats for boosting heat loss by evaporation from the skin (sweating) and by respiration (panting).

The total water evaporation calculated by subtracting water losses in milk, urine, and feces from water input (water intake + water in food) was 3 times greater (Table 3.3) in HS goats than TN goats (+2.23 L/d; $P < 0.01$). We were unable to distinguish between evaporation by

sweating and panting, but increased sweating rates have been reported in heat-stressed dairy cows (Shwartz et al., 2009) and goats (Baker, 1989).

Table 3.3. Water input and water losses of dairy goats under thermal neutral (TN, n = 8) and heat stress (HS, n = 8) conditions at late lactation (values are least squares means and SE of the difference).

	Treatment			Effect ($P <$)		
Item	TN	HS	SED	Treatment	Period	T × P[3]
Water intake, mL[1]	5,504	9,728	1,863	0.035	0.859	0.638
Water in food, mL	143	127	7	0.034	0.010	0.083
Water in milk, mL[1]	969	1,004	62	0.547	0.157	0.293
Urine volume, mL[1]	2,143	4,757	1,737	0.410	0.993	0.796
Water in feces, mL	1,426	825	144	0.002	0.326	0.178
Evaporation water, mL[1, 2]	1,074	3,304	1,430	0.007	0.442	0.476

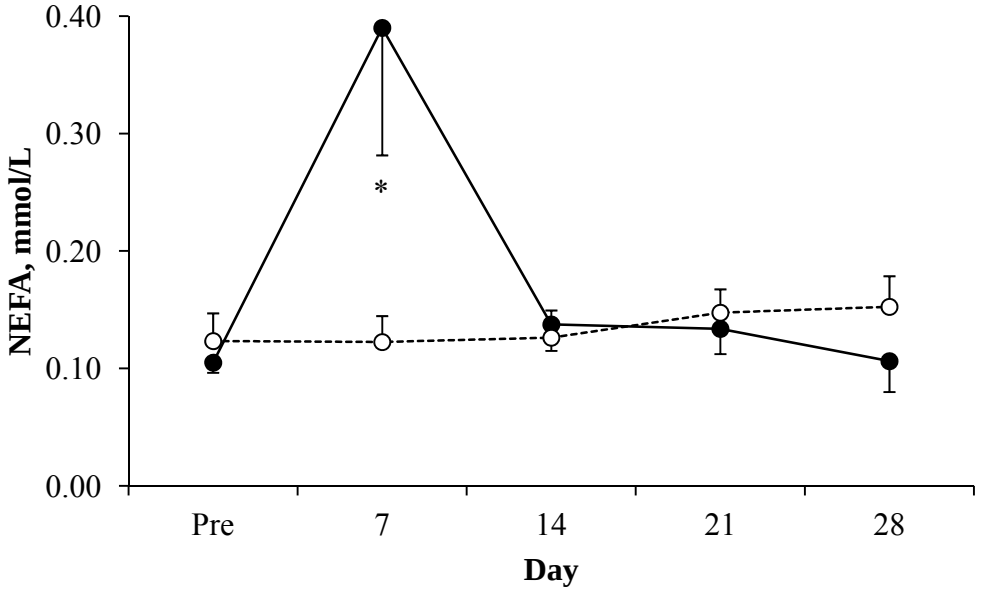
[1]P values extracted after log transformation because of the non-normal distribution of data.[2]Calculated by the difference between water input (water intake + water in food) and water losses in milk, urine, and feces without taking into account the water produced metabolically.[3]Interaction Treatment × Period.

3.4.4 Milk Yield

Despite the reduced DMI, increased body temperature, and the known negative effect of HS on milk production in dairy cows (West, 2003), milk yield and FCM did not vary between HS and TN goats (Table 3.2 and Figure 3.2; $P > 0.05$). Brown et al. (1988) reported that the exposure of dairy goats to constant conditions of 34ºC and 25% relative humidity (THI = 79) for 5 wk depressed milk yield in Alpine but not in Nubian goats.

Even when DMI was at its lowest value during the first wk, milk yield was not affected in HS goats. During wk 1, HS goats may have been able to partially cover their lactation requirements by body fat mobilization as indicated by the greater values of NEFA in plasma at d 7 of heat stress (Figure 3.3). From d 14 to 28 blood NEFA levels were similar between groups, but our late lactation HS goats were probably able to maintain milk yield because they partially recovered DMI, tended to have greater digestibility (see later), and had lower metabolic demands compared to early lactating goats. Milk yield response of dairy goats to HS in early lactation needs further research.

Figure 3.3. Plasma NEFA concentrations of dairy goats under thermal neutral (○, TN; n = 8) or heat stress (●, HS; n = 8) conditions at late lactation. Values are means with SE indicated by vertical bars (* indicates a difference at $P < 0.001$ between TN and HS treatments).



Our results of NEFA concentrations from d 14 to 28 (but not at d 7) agreed with findings obtained in dairy cows, where HS did not cause an increase in blood NEFA despite the reduced feed intake (Rhoads et al., 2009; Baumgard and Rhoads, 2013). Thus, it seems HS cows are more sensitive to insulin than TN, allowing a potent antilipolytic action of insulin that will prevent body fat mobilization. Consequently, lactating HS cows fail to have sufficient glucose for milk synthesis, and therefore milk yield was depressed. We did not observe such a decrease in milk yield in our HS goats, probably because the glucose was sufficient for the milk yield level at late lactation (see later).

3.4.5 Milk Composition

Milk TS and fat contents did not vary ($P > 0.05$) between HS and TN goats (Table 3.2). In short-term studies carried out using climate-controlled heat stress, milk fat was also not affected in cows (Rhoads et al., 2009; Shwartz et al., 2009) despite the known negative effect of summer on milk fat (Kadzere et al., 2002). This usual decrease in milk fat content of heat-stressed dairy cows during summer might be related to a reduction in forage intake, which could decrease the forage to concentrate ratio in the diet. In the current study, concentrate

amount was adjusted according to the forage intake in HS goats to maintain a constant forage to concentrate ratio throughout the experiment and similar to that of TN goats.

With the exception of milk NPN, content of protein and protein fractions in milk (true protein, CN, and whey protein) were reduced by heat stress (Table 3.2). Nevertheless, CN to protein ratio was not affected. Similarly, dairy cows under controlled conditions of HS decreased their milk protein content (Rhoads et al., 2009; Shwartz et al., 2009). Decreased protein intake and increased sweat secretion that contains protein and urea (Joshi et al., 1968) might have limited the availability of AA for milk protein synthesis. Moreover, the decrease in milk protein content under HS may be due to a lowered microbial protein synthesis in the rumen because of changes in rumen environment (dilution or clearance of soluble substances) by the high water intake. Huber et al. (1994) indicated that under decreased feed intake conditions, increasing the percentage of rumen undegradable protein and supplementing with lysine increased milk protein content under hot conditions in dairy cows. On the other hand, Bernabucci et al. (2002) suggested that decreased mammary synthesis of milk protein (rather than the reduction in AA intake) is the reason for the low milk protein during the hot season in dairy cows.

3.4.6 Digestibility and Nitrogen Balance

Goats under HS showed numerically greater values of digestibility and tended to have greater ADF digestibility than TN (+3.1 points; $P< 0.10$), as shown in Table 3.4. To our knowledge, no data are available comparing digestibility under TN and HS ambient conditions in lactating dairy goats. Our results agree with those of previous research carried out under climatic chamber conditions with male goats (Hirayama et al., 2004), dairy cows (McDowell et al., 1969) and heifers (Bernabucci et al., 1999). The increased digestibility in the HS treatments in the aforementioned studies might be partially due to the reduction of DMI as also observed for HS goats in our results (Table 3.2). Another reason for the enhanced digestibility under HS conditions could be a depressed passage rate of the solid phase of digesta as reported by Bernabucci et al. (1999). On the other hand, a greater rate of passage of the liquid phase of the digesta may be expected as a consequence of the dramatic increase in water intake. This could have an impact on the availability of rumen soluble fermentable compounds, although this statement needs experimental confirmation. The tendencies observed for an increased digestibility of nutrients (i.e., DM, OM and ADF; Table 3.4) in the HS goats might have compensated the reduction in DMI, and could partially explain the lack of effects of HS on milk yield in our results.

Table 3.4. Digestibility coefficients and nitrogen balance of dairy goats under thermal neutral (TN, n = 8) and heat stress (HS, n = 8) conditions at late lactation (values are least squares means and SE of the difference).

	Treatment			Effect (P <)		
Item	TN	HS	SED	Treatment	Period	T × P[1]
Digestibility, %						
DM	56.6	58.8	2.3	0.121	0.567	0.047
OM	58.8	61.2	1.4	0.109	0.408	0.058
CP	70.5	72.1	1.5	0.306	0.723	0.021
NDF	36.0	38.8	1.9	0.157	0.985	0.765
ADF	35.1	38.2	1.7	0.094	0.143	0.600
N balance						
Intake, g/d	48.4	42.1	0.8	0.001	0.012	0.059
Fecal excretion, g/d	14.3	11.8	0.8	0.010	0.351	0.017
Urinary excretion, g/d	20.3	16.4	1.3	0.012	0.962	0.268
Apparent absorption, %	70.5	72.1	1.5	0.306	0.725	0.021
Retention, g/d	13.9	13.9	1.0	0.950	0.162	0.057

[1]Interaction Treatment × Period.

Although HS goats had lower N intake (Table 3.4), as a consequence of the DMI reduction, they experienced lower N losses in feces and urine, which resulted in similar daily N retention to TN goats (13.9 ± 1.0 g/d on average). Despite the similar N retention, milk protein content was lower in HS than in TN goats, indicating that ingested N would have been directed to another metabolic functions rather than milk protein synthesis in HS goats. It is possible that a portion of N intake was lost in sweat in the form of urea as previously proposed by Joshi et al. (1968). Moreover, the possibility of a lower supply of essential AA for milk protein synthesis could not be excluded.

Treatment by period interactions were detected for DM and CP digestibilities, and for fecal excretion and apparent absorption of N (Table 3.4). These significant interactions could indicate some carryover effect when HS goats went to the TN treatment in the second period. However, this seems unlikely in our case as these interactions might result from the effect of period on DMI (P < 0.01; Table 3.2), where the DMI reduction in HS goats was more pronounced during period 2 compared to period 1 (data not shown).

3.4.7 Blood indicators and Urinary pH

Heat stress had no effect on blood glucose, urea, hematocrit and hemoglobin concentrations (Table 3.5; $P > 0.05$). A tendency in the treatment × hour interaction was detected for blood glucose ($P < 0.10$). At 0800 h, HS goats showed lower glucose concentration than TN goats ($P < 0.05$), while concentration at 1700 h increased ($P < 0.001$) and was similar to that of TN goats. It must be stressed that the morning blood samples were taken 1 h before offering the diet, whereas the afternoon sampling was done 1 h after distribution of the second portion of concentrate, which may have contributed to the similar blood glucose levels between groups at 1700 h.

Blood pH is regulated by a complex system of buffers that continuously work to maintain it slightly basic in a range of 7.35 to 7.45 in most mammals (Constable, 1999). Measured blood pH was similar at 0800 in TN and HS goats, but slightly decreased at 1700 h ($P < 0.05$) in the TN goats. Despite the importance of blood pH for understanding the mechanism of respiratory evaporative heat loss, changes observed in our goats were marginally relevant and varied within the normal range in both TN and HS groups.

Values of total CO_2, pCO_2, HCO_3^-, and base excess were lower in HS compared to TN goats (Table 3.5; $P < 0.01$). The deceased pCO_2 and HCO_3^- under heat stress conditions agree with the results reported in dairy cows by Schneider et al. (1988). The greater respiration rate observed in panting HS goats contributed to a greater loss of CO_2, lowering the carbonic acid content of the blood. As a consequence, HCO_3^- was transferred from blood to urine by the kidney for maintaining blood pH constant. Heat stress had no effect on blood Na and K concentrations in accordance with results previously reported in HS dairy cows (Schneider et al., 1988). On the other hand, Cl concentration was greater, at both time points, in HS than in TN goats (Table 3.5; $P < 0.05$). Calamari et al. (2007) reported an inverse relationship between blood HCO_3^- and Cl in dairy cows under TN and HS conditions. Due to the greater Cl concentrations of the HS goats, they also have greater anion gap values, compared to TN goats (Table 3.5; $P < 0.05$). It was expected that the increased HCO_3^- secretion in the urine of HS goats should raise the urine pH, but we observed the opposite as urine pH of HS goats tended ($P = 0.108$) to be lower than TN goats. We speculated that HS goats were able to increase their renal excretion of H+, which resulted in a partial re-absorption of HCO_3^- into the blood as previously observed by Masero and Siegel (1977). In fact, the decrease in pCO_2 at 1700 h due to HS was more marked (-20%) than the decrease in HCO_3^- (-12%).

Table 3.5. Metabolic and acid-base balance indicators of dairy goats under thermal neutral (TN, n = 8) and heat stress (HS, n = 8) conditions at different daily hours at late lactation (values are least squares means and SEM).

	TN		HS			Effect (*P*<)		
Item	0800 h[1]	1700 h[2]	0800 h[3]	1700 h[3]	SEM	Treatment	Hour	T × H[4]
Glucose, g/L	3.21	3.41	3.04	3.44	0.05	0.197	0.001	0.057
Urea, mg/dL	40.4	30.0	36.5	28.3	2.5	0.331	0.001	0.498
Na, mmol/L	139.5	140.4	142.3	141.0	1.2	0.161	0.881	0.403
K, mmol/L	3.83	4.16	3.70	4.25	0.10	0.793	0.003	0.399
Cl, mmol/L	105.0	108.9	108.3	110.4	0.9	0.031	0.004	0.324
Hematocrit, %PCV[5]	18.63	17.75	18.25	16.63	0.63	0.372	0.003	0.296
Hemoglobin, mmol/L[6]	6.33	6.03	6.23	5.64	0.21	0.363	0.002	0.187
pH	7.42	7.38	7.42	7.42	0.01	0.156	0.142	0.056
Total CO_2, mmol/L[6]	26.9	24.4	22.3	21.5	0.8	0.002	0.036	0.231
Anion gap, mmol/L[6]	12.50	12.00	16.50	14.00	0.73	0.001	0.099	0.428
pCO_2, mm Hg	38.9	39.8	33.1	31.5	1.7	0.006	0.769	0.300
HCO_3^-, mmol/L[6]	25.71	23.41	21.29	20.51	0.83	0.003	0.041	0.283
Base excess[6]	1.38	–1.75	–3.00	–4.00	0.87	0.005	0.019	0.193
Urine pH	9.09	8.94	8.91	8.85	0.08	0.108	0.215	0.559

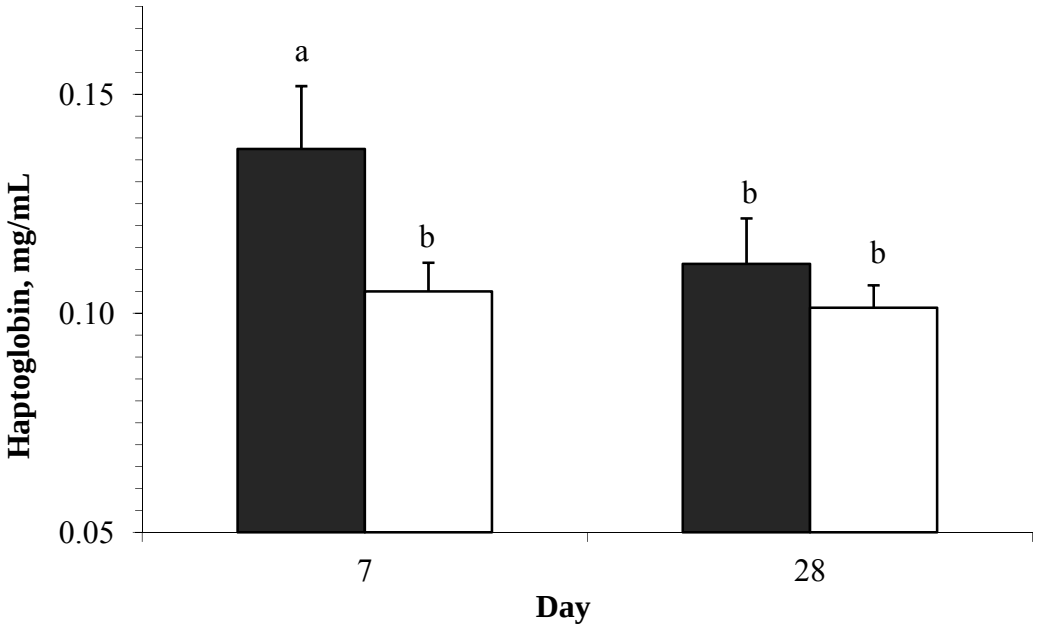
[1]Before changing from night (30.5°C and 40% humidity, THI = 77) to day (37°C and 40% humidity, THI = 85) conditions.[2]During the day (37°C and 40% humidity, THI = 85) conditions. [3]Indoorsdaily variation from 15 (night) to 20°C (day) at 40% relative humidity (THI = 59 to 65).[4]Treatment × hour interaction.[5]Packed cell volume.[6]Calculated values by the i-STAT device software.

The secretion of HCO_3^- in urine and its re-absorption suggests a large requirement and turnover of body bicarbonate to maintain blood pH during heat stress.

3.4.8 Haptoglobin Concentration in Blood

Haptoglobin, an acute phase protein linked to metabolic stress, concentration in blood plasma was greater in HS than TN at d 7 ($P < 0.05$), when effects of HS were more marked, but differences between treatment groups disappeared at d 28 (Figure 3.4; $P > 0.05$).

Figure 3.4. Haptoglobin concentrations in plasma of dairy goats under thermal neutral (□, TN; n = 8) or heat stress (■, HS; n = 8) conditions at late lactation. Values are means with SE indicated by vertical bars. [a, b] Means with different letters differ ($P < 0.05$)**.**



Ametaj et al. (2005) indicated an association between increased serum haptoglobin concentration and hepatic lipidosis in periparturient dairy cows. Hiss et al. (2009) found that elevated haptoglobin concentrations in milk of dairy cows were associated with high NEFA values in early lactation. This relationship was also observed in our HS goats.

It seems that HS goats at d 7 responded by increased circulating haptoglobin when they were metabolically challenged as evidenced by reduced feed intake (Figure 3.2) and greater NEFA concentrations (Figure 3.3). However, when goats were more adapted to heat stress conditions (i.e. d 28), haptoglobin levels returned to values similar to TN goats.

3.4.9 Corticosterone in Feces

Fecal corticosterone has been used to evaluate stress in cows (Morrow et al., 2002). This approach is based on the fact that glucocorticoids are secreted by the adrenal gland after the activation of the hypothalamic-pituitary-adrenal axis by a stressor. Circulating glucocorticoids are metabolized (conjugated) in the liver and excreted via the urine and feces.

Corticosterone concentrations in feces did not vary between TN and HS goats and averaged 4.28 ± 0.55 ng/g DM. Similarly, HS did not increase plasma cortisol concentration in cows (El-Nouty et al., 1978) and goats (Olsson and Dahlborn, 1989). Nevertheless, it should be stressed that fecal samples were collected at d 35, and it is possible that differences between TN and HS goats would have been detected if fecal corticosterone was measured earlier (i.e. at d 7 when HS goats were suffering greater metabolic stress).

3.5 CONCLUSIONS

Despite the reduction observed in feed intake of heat-stressed goats, they produced similar milk yield to goats under thermal neutral conditions at late lactation. However, milk protein content decreased in the heat-stressed goats with no change in milk fat content. Heat-stressed goats had similar N retention to goats under thermal neutral conditions, indicating that the ingested N might have been directed to another metabolic functions rather than milk protein synthesis.

Further studies are needed to test the heat stress effects during early lactation and to clarify whether reduced milk protein content is related to a nutrient limiting factor or reduced mammary protein synthesis as well as to evaluate the effects on coagulation properties of the milk.

CHAPTER 4

Effects of heat stress on lactating dairy goats: metabolism and changes in behavior

CHAPTER 4

Effects of heat stress on lactating dairy goats: metabolism and changes in behavior

4.1 ABSTRACT

Heat stress (HS) induces hormonal and behavioral changes, but little is known about these changes in dairy goats. Eight multiparous Murciano-Granadina dairy goats (43.3 ± 1.6 kg BW; 2 ± 0.04 L/d; 81 ± 3 DIM) were kept in metabolic cages and randomly assigned to 2 climatic treatments according to a crossover design (two 28-d periods). Treatments were:1) thermal neutral (TN; 15 to 20°C, 40 to 45% humidity, THI = 59 to 65), and 2) heat stress (HS, 12 h/d at 37°C and 40%, and 12 h/d at 30°C and 40%, THI = 86 and 77, respectively). Jugular silicon catheters werefitted, and insulin challenge, epinephrine challenge and glucose tolerance test were done on different days. The insulin (4.6 µg/kg BW), epinephrine (2 µg/kg BW) and glucose (0.25 g/kg BW) solutions were administrated via the jugular catheter. Blood samples were collected at −30, −20, −10, 0, 5, 10, 20, 30, 45, 60, 90, and 120 min relative to the administration for analysis of plasma insulin, NEFA and glucose concentrations. Moreover, 8video cameras with infrared illuminator were installed on the top of each cage. All the experimental days (28 d × 2 periods) were filmed, but only the 3rdd of each period was analyzed for each goat. Changing the position bouts, duration of remaining standing, and eating and drinking bouts and duration were measured. Goats in both groups had similar blood NEFA after insulin injection, but NEFA values were greater ($P< 0.05$) in HS than TN goats after epinephrine administration. The HS goats secreted lower ($P< 0.05$) amounts of insulin than TN goats in response to the glucose tolerance test. Furthermore, TN and HS goats had similar eating bouts, but the duration of each bout was lower in HS than in TN. On the other hand, HS had greater number of drinking bouts with no change in drinking bout durations between both groups. In conclusion, body lipid tissue of HS goats became more resistant to lipolysis, making goats unable to mobilize body fat reserves despite the negative energy balance. Moreover, the typical reduction of feed intake in HS is due the shorter time of eating bouts, whereas the greater water consumptions is explained by the increment in drinking bouts.

4.2 INTRODUCTION

Numerous physiologic mechanisms for coping with heat stress (HS) have been reported in different dairy animal species (Blackshaw et al., 1994; Baumgard and Rhoad, 2013; Salama et al., 2014). Greater sweating, higher respiration rate, vasodilation with increased blood flow to skin surface, reduced metabolic rate, decreased DM intake and altered water metabolism are the physiologic responses to HS that might explain the negative impact of HS on milk production. Moreover, circulating T3 and T4 decline by up to 25% (Magdub et al., 1982; Beede and Collier, 1986; Silanikove et al., 1992), which is consistent with the decrease in metabolic rate, feed intake, growth and milk production under HS (Beede and Collier, 1986).

In cows, one characteristic response to negative energetic balance is a reduction in circulating insulin coupled with a reduction in systemic insulin sensitivity. The reduction in insulin action allows for adipose lipolysis and mobilization of non-esterified fatty acids (NEFA) (Bauman and Currie, 1980). Post-absorptive carbohydrate metabolism is also altered by the reduced insulin action during the negative energy balance with the net effect of reduced glucose uptake by systemic tissues (i.e. muscle and adipose). The reduced nutrient uptake coupled with the net release of nutrients (i.e. amino acids and NEFA) by systemic tissues are key mechanisms implemented by cows during negative energy balance to support lactation (Bauman and Currie, 1980). However, in case of HS-dairy cows (Rhoads et al., 2009; Shwartz et al., 2009), it has been shown that despite the reduced feed intake and the negative energy balance, animals had no increase in plasma NEFA, and this agrees with other heat-stressed ruminant models (Sano et al., 1983; Itoh et al., 1998; Ronchi et al., 1999; Hamzaoui et al., 2013a). The lack of an elevated NEFA response is especially surprising, as acute HS causes a marked increase in circulating hormones as cortisol, norepinephrine and epinephrine levels (Collier et al., 2005). These hormones act as catabolic signals that normally stimulate lipolysis and adipose mobilization. This is also surprising as calculated energetic balance is traditionally thought to be closely associated with circulating NEFA levels. Hormonal challenges and glucose tolerance test could allow the understanding of the metabolic changes by HS.

On the other hand, HS could compromise the animal welfare because HS represents a situation in which the animal has difficulty in coping with its environment. Farm animals in central and western Spain, or in the southern areas of France, Italy and Greece, are exposed annually for 3–5 mo to considerable HS (Silanikove et al., 1992). Despite the numerous

published studies on the impact of HS on productive and reproductive parameters, little is known about the changes in animal behavior due to HS, especially in dairy goats.

In the current study, mid-lactation dairy goats kept under HS were used to evaluate the response to insulin, epinephrine and glucose challenges. Moreover, video cameras were used to record goats behavior (eating, drinking, position changes) throughout the day.

4.3 MATERIALS AND METHODS

4.3.1 Animals and Management Conditions

Animal care conditions and management practices agreed with the procedures stated by the Ethical Committee of Animal and Human Experimentation of the Universitat Autonoma de Barcelona and the codes of recommendations for the welfare of livestock of the Ministry of Agriculture, Fisheries and Food of Spain (MAPA, 2010).

Eight multiparous Murciano-Granadina dairy goats in mid-lactation (43.3 ± 1.6 kg BW; 2 ± 0.04 L/d, 81 ± 3 DIM) were used from the herd of the experimental farm of the Universitat Autonoma of Barcelona. Goats were divided into 2 balanced groups of 4 and maintained in metabolic cages on 2 different environmental conditions: thermal neutral [TN; 15 to 20°C, 40-45% humidity (temperature humidity index, THI = 59 to 65)], and heat stress [HS; 37°C, 40% humidity (THI = 86) from 0900 to 2100 h; and 30 °C, 40% humidity (THI = 77) from 2100 to 0900 h].Under both conditions, goats had a 12:12 h light:dark cycle. The experimental design was a crossover design with 2 periods, lasting 28 d and 4 goats each. When goats were switched from TN to HS conditions, a transition period of 2 d was allowed (1 d at 25°C, 1 d at 30°C), but the change from HS to TN was abrupt. Goats had a 4-wk pre-experimental period under TN conditions for the adaptation to the diet and metabolic cages.

Data of environmental temperature and humidity were recorded every 10 min throughout the experiment by a data logger (DL), (Opus 10, Lufft, Fellbach, Germany). The THI values were calculated according to NRC (1971) as follows:

$THI = (1.8 \times T_{db} + 32) - [(0.55 - 0.0055 \times RH) \times (1.8 \times T_{db} - 26.8)]$, where T_{db} is the dry bulb temperature (°C) and RH is the relative humidity (%).

Goats were fed TMR (alfalfa hay, 70%; ground barley grain, 14.4%; corn flour, 8.4%; soybean meal, 2.5%; soybean hulls, 4.3%; molasses, 0.3%; salt, 0.01%; sodium bicarbonate, 0.03%; carbonate, 0.02%; dicalcium phosphate, 0.01%; calcium carbonate, 0.01%; CVM for goats, 0.02%). Chemical composition and nutritive value of the TMR are shown in Table 4.1.

Table 4.1. Chemical composition and nutritive value (DM basis) of the total mixed ration (TMR) ingredients used for dairy goats.

Item	Total mixed ration
Component, %	
Dry matter	89.31
Organic matter	89.87
Crude protein	17.50
Neutral detergent fiber	43.80
Acid detergent fiber	27.00
Nutritive value[1]	
UEm,[2] /kg	0.83
UFL,[3] /kg	0.83
NE_L, Mcal/kg	1.40
PDIE,[4] g/kg	97
PDIN,[5] g/kg	110
PDIA,[6] g/kg	45
Ca, g/kg	10.60
P, g/kg	2.40

[1] Calculated according to Institut National de la Recherche Agronomique (INRA, 2007).[2] Fill units for sheep (1 UEm = 1 kg of reference grass DM).[3] Feed units for lactation (1 UFL = 1.7 Mcal of NEL).[4] Protein digested in the small intestine supplied by microbial protein from rumen-fermented OM.[5] Protein digested in the small intestine supplied by microbial protein from RDP.[6] Protein digested in the small intestine supplied by RUP.

Moreover, mineral and vitamin blocks were freely available (Na, 16%; Ca, 12%; bicarbonate and seaweed, 12%; P, 5.5%; Mg, 2.2%; Zinc oxide, 2,000 mg/kg; manganese sulfate, 1,000 mg/kg; potassium iodide, 60 mg/kg; Cobalt, 40 mg/kg; iron sulfate, 40 mg/kg; sodium selenite, 15 mg/kg; yeasts and S. Cerevisiae, 10 mg/kg; vitamin A, 120,000 IU/kg; vitamin D3, 32,000 IU/kg; vitamin E, 120 mg/kg).

Goats were milked once daily (0800 h) with a portable milking machine (Westfalia-separator Ibérica, Granollers, Spain) with recorded jars (3 L ± 5%). Milking was conducted at a vacuum pressure of 42 kPa, a pulsation rate of 90 pulses/min, and a pulsation ratio of 66%. The milking routine included cluster attachment without udder preparation or teat cleaning, machine milking, machine stripping before cluster removal, and teat dipping in an iodine solution (P3-ioshield, Ecolab Hispano-Portuguesa, Barcelona, Spain).

Daily rectal temperatures and respiration rates were recorded at 0800, 1200, and 1700 h. Feed intake, water consumption, milk yield were recorded daily throughout the experiment. Moreover, milk samples were collected weekly for milk composition evaluation.

4.3.2 Hormonal challenges and glucose tolerance test

Jugular silicon catheters (Vygon. B.P. 7-95440, Ecouen. France) were inserted in the 3rd wk of the 2nd period, and insulin challenge, epinephrine challenge and glucose tolerance test were done on different days after milking and before the morning meal throughout the 4th wk. The insulin (4.6 μg/kg BW), epinephrine (2 μg/kg BW) and glucose (0.25 g/kg BW) solutions were administrated via the jugular catheter and immediately followed by 10 mL sterile solution. Blood samples were collected at −30, −20, −10, 0, 5, 10, 20, 30, 45, 60, 90, and 120 min relative to the administration. Blood samples were collected by syringe into glass tubes containing 250 units of sodium heparin and were immediately placed on ice. After centrifugation of whole blood for 15 min at 1,500 × *g* and 4°C, plasma was divided into different aliquots and stored at −20°C for subsequent analysis of plasma insulin, NEFA and glucose concentrations.

The NEFA were determined by the colorimetric enzymatic test ACS-ACOD method using a commercial kit (Wako Chemicals GmbH, Neuss, Germany). Insulin was determined by solid phase two-site enzyme immunoassay based on the direct sandwich technique using the Mercodia Ovine Insulin ELISA kit (Mercodia AB, Uppsala, Sweden). Glucose was determined by Trinder method using Glucose GOD-PAP kit (Biolabo SA, Maizy, France).

4.3.3 Continuous recording video

Eight digital color cameras (model VCAM—420CA, Circontrol1, Barcelona, Spain), with a focal lens (model LTC 0500/50, Philips, Eindhoven, the Netherlands), fitted with infrared illuminator were used and one camera installed in front of each goat on the top of each cage (picture 4.1). All the experimental days (28 d × 2 periods) were filmed, resulting in a total of 672 h of video / goat / period. Videos were digitized and stored on SATA (Serial Advance Technology Attachment) hard disks of 500 GB each. The videos were watched using a digital recorder (VDVR-9NX Circontrol1, Barcelona, Spain) with screen and remote control, which allowed the manual control of the video. Due to the accuracy of the recorder, the images could be displayed at a standard frame rate of 25 pictures per second.

Picture 4.1. Dairy goats in the metabolic cages fitted with the video cameras in thermo-neutral (top) and heat stress (bottom) conditions.

The following 6 behavior indices were measured for each goat:

1) Time of remain standing, as the time duration spent by the goat standing for different activities (eating, drinking or idling).

2) Times of changing the position, describes how many times the goat changes its position from standing to lying down or vice versa.

3) Eating bouts, describes how many times the goat visits the feeder and start eating.

4) Eating time, describes the duration taken by the goat eating from the feeder.

5) Drinking bouts, describes how many times the goat visits the water trough and start drinking.

6) Drinking time, describes the duration taken by the goat drinking from the water trough.

According to our previous observations (Hamzaoui et al., 2013a) and the current study (see later), goats were suffering the maximum after 48-72 h of HS exposure. Therefore, the 3rdday of each treatment (TN, HS) and each period was chosen to obtain the behavior parameters. The activity throughout the day was considered as diurnal (12 h daylight from 900 to 2100 h) and nocturnal (12 h dark from 2100 to 900). The recorder with screen and the remote control were used to watch and manipulate the videos manually. All the data was written down on Excel sheet; e.g., when the goat starts to eat time was recorded and when it finishes time was recorded, and by the difference in time we obtained the meal bout duration. Time needed to watch 24 h of one goat was 4 h. Consequently, the total watching session time was 8 goats × 2 periods × 4 h = 64 h.

4.3.4 Statistical Analyses

Data were analyzed by the PROC MIXED for repeated measurements of SAS version 9.1.3 (SAS Institute Inc., Cary, NC). The statistical mixed model of video recording data (from around 3000 lines of data in Excel sheet, a dynamic table was generated in order to make easier the manipulation of data) contained the fixed effects of the treatment (HS vs. TN), daytime (daylight vs. night), and period (1 vs. 2); the random effect of the animal; the interactions treatment × daytime and treatment × period; and the residual error. The model took into account the possible carryover effects of previous HS periods through the treatment × period interaction. The statistical mixed model of hormonal challenges contained the fixed effects of the treatment (HS vs. TN), time relative to injection, and period; the random effect

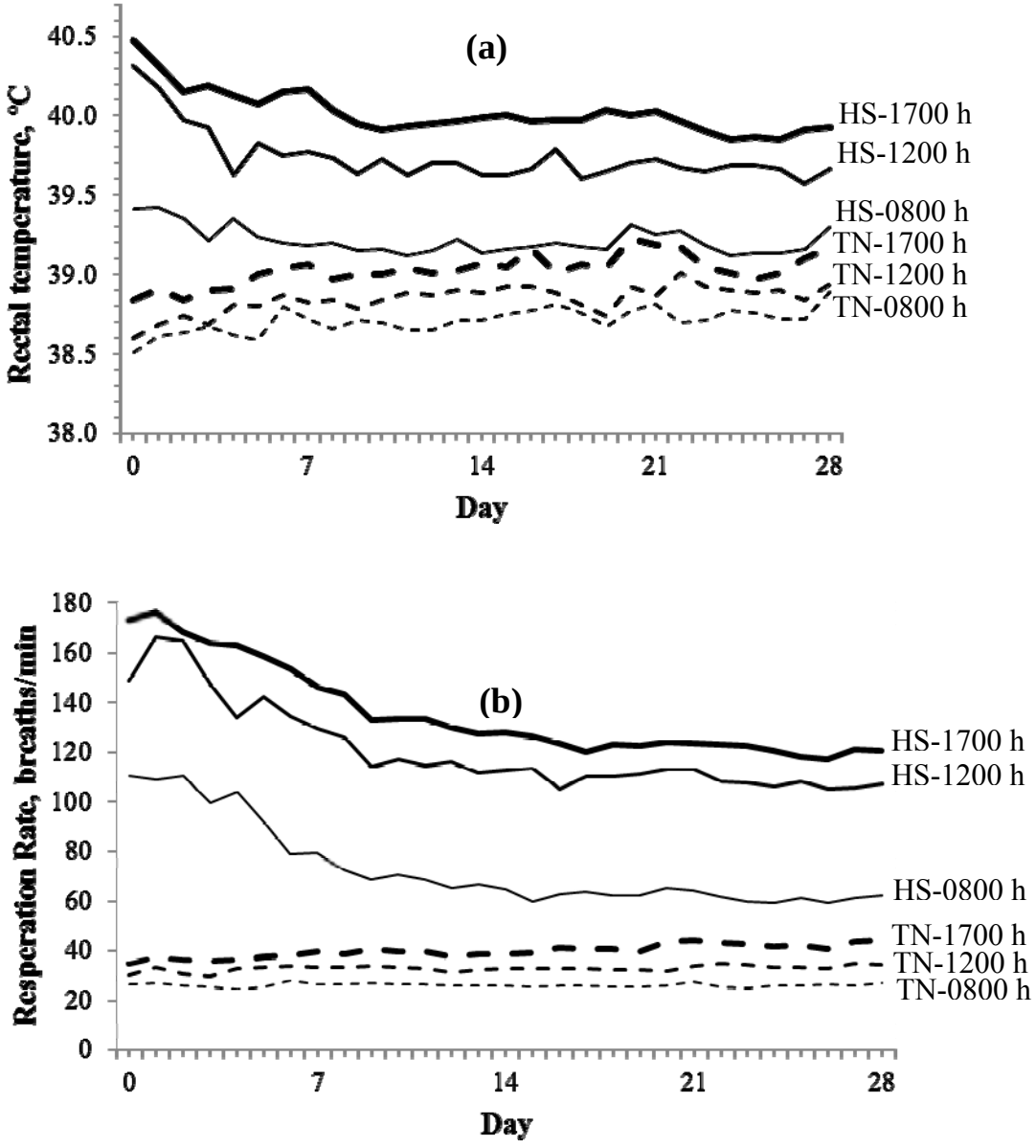
of the animal; the interactions treatment × period and treatment × time relative to injection; and the residual error.

4.4 RESULTS AND DISCUSSION

4.4.1 Lactational Performances

Rectal temperatures and respiration rates increased ($P < 0.01$) from 0800 to 1700 h in both goat groups, and were greater ($P < 0.001$) in HS than in TN goats (Figure 4.1).

Figure 4.1. Rectal temperature (a) and respiration rate (b) throughout the day (8, 12, and 17 h) in dairy goats under thermal neutral (TN; n = 8) and heat stress (HS; n = 8) conditions.



Goats under HS had the maximum values of rectal temperature and respiration rate during the first 2-3 d and then decreased ($P < 0.05$), but remained greater ($P < 0.001$) than the TN goats.

The DMI decreased ($P < 0.001$) by -29% as a result of HS treatment (Table 5.2). The reduction in feed intake by HS was maximum at d 5 (-36%) and partially started to recover thereafter (data not shown), but always was lower ($P < 0.001$) than in TN goats. Compared to TN, HS goats had lower ($P < 0.05$) milk yield, milk fat, milk protein, and milk lactose. These results agree with what obtained in dairy cows (West, 2003; Rhoads et al., 2009) and Alpine goats (Brown et al., 1988). However, losses in milk yield and milk components in the current study are greater than what previously observed in the same breed at late lactation (Hamzaoui et al., 2013a). It seems that the impact of HS varies according the stage of lactation, being greater at earlier stages of lactation (current study) than at late lactation (Hamzaoui et al., 2013a).

Table 5.2. Performances of Murciano-Granadina dairy goats under thermal neutral (TN, n = 8) and heat stress (HS, n = 8) conditions. Values are LSM and SE of the difference (SED).

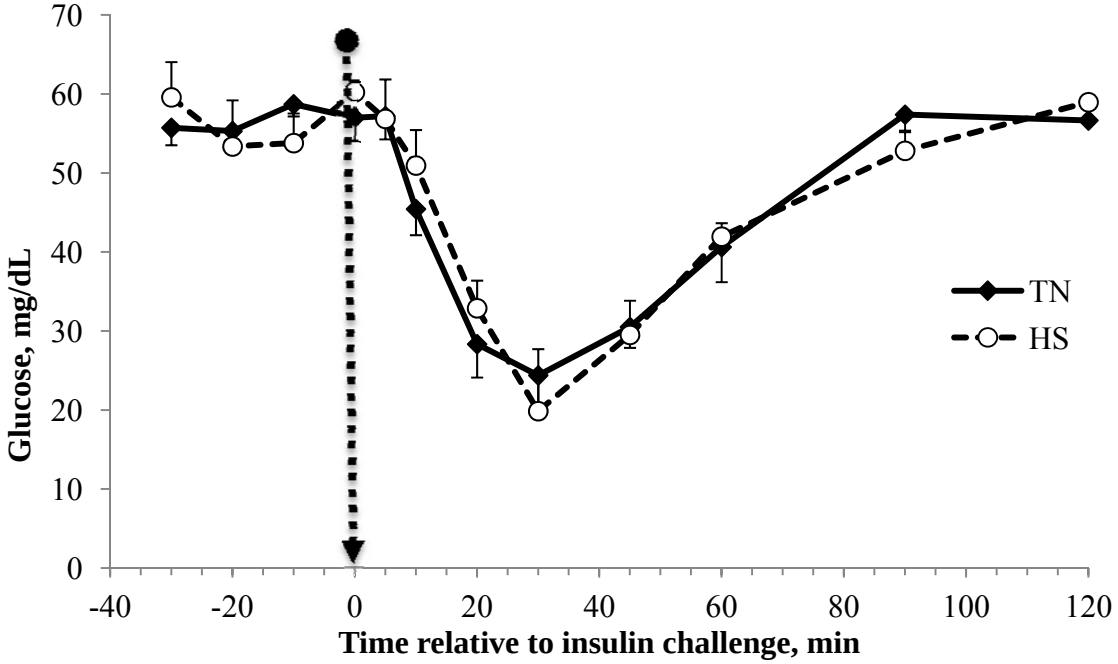
Item	Treatment			Effect (*P*- Value)		
	TN	HS	SED	Treatment	Period	T x P[1]
DMI, Kg/d	2.49	1.77	0.04	0.001	0.010	0.767
water consumption, L/d	5.9	10.0	0.34	0.001	0.912	0.380
Milk yield, L/d	1.70	1.56	0.15	0.001	0.001	0.729
3.5 % FCM[2], L/d	1.90	1.62	0.02	0.001	0.001	0.744
Milk composition, %						
Total solids	12.9	11.8	0.11	0.001	0.368	0.687
Fat	4.26	3.76	0.10	0.001	0.730	0.510
Protein	3.74	3.26	0.09	0.001	0.230	0.260
Casein	3.20	2.83	0.08	0.001	0.010	0.895

[1]Interaction of treatment (T) × period (P).[2]3.5% FCM = L of milk yield × [0.432 + 0.162 × (fat %)].

4.4.2 Hormonal challenges and glucose tolerance test

The glucose basal levels averaged 58.6 ± 5.6 mg/dL and were similar in the plasma of TN and HS goats (Figure 5.2). The level of plasma glucose decreased after insulin (lipogenic signal) administration and reached the lowest ($P < 0.001$) level at 30 min (22.2 ± 3.9 mg/dL).

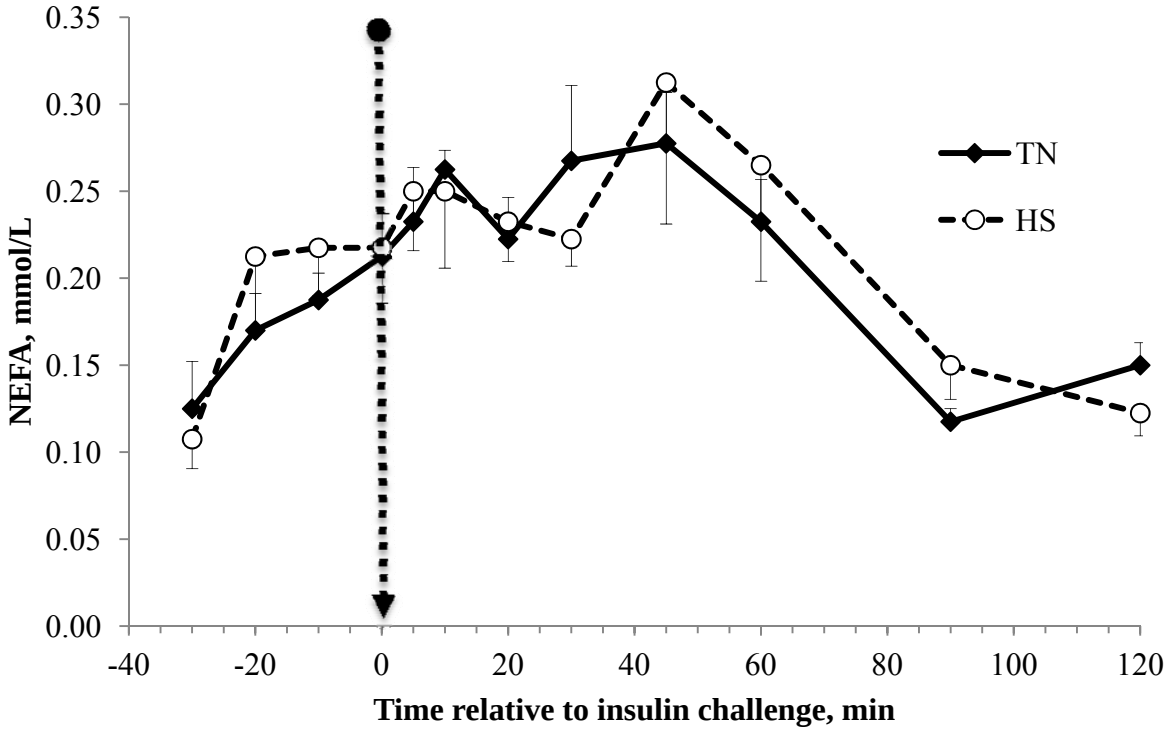
Figure 5.2. Glucose response to insulin challenge of dairy goats under thermal neutral (TN; n = 4) or heat stress (HS; n = 4) conditions. Discontinued arrow indicates time of insulin injection. Values are means with SE indicated by vertical bars.



Thereafter, blood glucose concentration increased gradually to basal levels at 90 min and remained unchanged until min 120. The response in blood glucose to insulin injection was similar for TN and HS goats.

The basal NEFA levels averaged 0.21 ± 0.03 mmol/L before the insulin injection and did not vary between TN and HS goats, confirming the no body fat reserves mobilization by HS (Figure 4.3). As for the response in glucose, the response of NEFA to insulin administration was similar in TN and HS goats. Despite the reduction in feed intake and body weight under HS, that response was not accompanied by body fat mobilization as blood NEFA levels did not vary between HS and TN goats. Fat mobilization was not observed under HS conditions in sheep (Achmadi et al., 1993), heifers (Itoh et al., 1998; Ronchi et al., 1999), cows (Rhoads et al., 2009; Shwartz et al., 2009), and goats (Sano et al., 1983; Hamzaoui et al., 2013a). Specifically in dairy cows, Rhoads et al. (2009) and Baumgard and Rhoads (2013) reported that blood NEFA did not vary due to HS (similar to our goats), but blood insulin levels were significantly increased (such an increase was not detected in dairy goats).

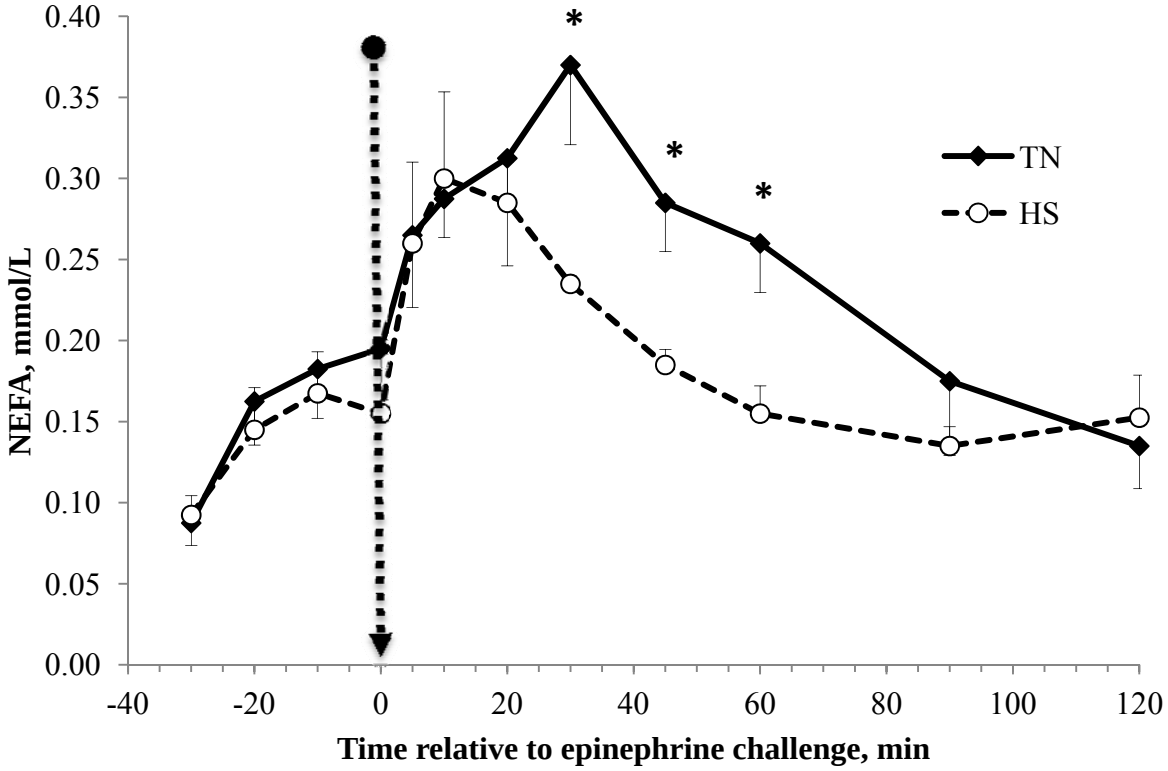
Figure 4.3. The NEFA response to insulin challenge of dairy goats under thermal neutral (TN; n = 4) or heat stress (HS; n = 4) conditions. Discontinued arrow indicates time of insulin injection. Values are means with SE indicated by vertical bars.



It is well known that insulin causes cells in the liver, skeletal muscles, and fat tissue to absorb glucose from the blood. In the liver and skeletal muscles, glucose is stored as glycogen, and in fat cells (adipocytes) it is stored as triglycerides. According to our results, it seems that this happens similarly in TN and HS goats.

The NEFA response of TN and HS goats following epinephrine administration (lipolysis signal) is shown in Figure 4.4. When goats were injected with epinephrine, an increase in blood NEFA was observed in both TN and HS goats. The peak of NEFA was observed at 10 and 30 min in HS and TN goats, respectively. Moreover, the peak was greater ($P< 0.05$) in TN than in HS goats. At 45 and 60 min after epinephrine administration, TN goats were still having greater ($P < 0.05$) blood NEFA levels. A previous study (Collier et al., 2005) showed that although HS is usually accompanied by higher levels of lipolytic hormones as cortisol, no increase in NEFA was detected. Results of the current study clearly indicate that HS induces an insensitivity of lipid body tissues to lipolytic hormones, making fat mobilization difficult when ambient temperature is high.

Figure 4.4. The NEFA response to epinephrine challenge of dairy goats under thermal neutral (TN; n = 4) or heat stress (HS; n = 4) conditions. Discontinued arrow indicates time of epinephrine injection. Values are means with SE indicated by vertical bars. * indicates a difference at $P < 0.05$ between TN and HS treatments.

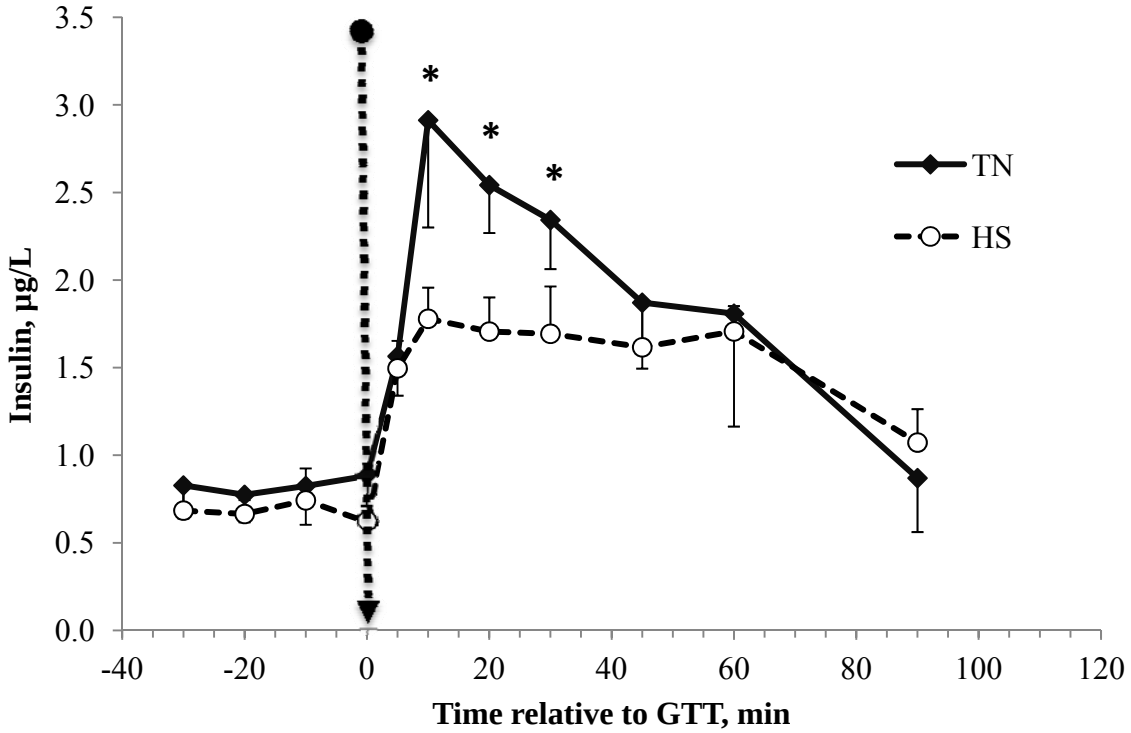


Insulin response of HS and TN goats following glucose administration is shown in Figure 4.5. Insulin peak was observed at 10 min in both goat groups, but the peak was greater ($P< 0.05$) in TN than in HS goats. This finding might indicate that the pancreas of HS goats is less sensitive, which could be a way to maintain normal glucose levels in blood under HS. Heat-stressed lactating goats (Sano et al., 1985; Hamzaoui et al., 2013a) and no lactating ewes (Sano et al., 1983) were able to maintain similar blood glucose levels to TN animals with no change in blood insulin concentration. Nevertheless, blood glucose significantly decreased by HS in dairy cows in accordance with the greater blood insulin (Rhoads et al., 2009; Baumgard and Rhoads, 2013).

Other mechanisms could explain the ability of HS-goats to maintain blood glucose. First, kidneys probably play an important role to maintain blood glucose under HS. Many studies conducted in animals, including ruminants, have provided considerable evidence that the mammalian kidney produce glucose and release it under various conditions (Gerich et al.,

2001). The greater levels of BHB in urine of HS goats (unpublished data) might indicate that ketone bodies arrived to kidneys and accelerated the gluconeogenesis. Although ketone bodies are weakly gluconeogenic, they are used by kidneys as a fuel of respiration to spare glucogenic substrates for gluconeogenesis (Krebs et al., 1965) and they activate the pyruvate carboxylase (a rate-limiting enzyme that controls lactate and alanine entry into the gluconeogenic pathway) to produce glucose as proposed by Kaufman and Bergman (1971) in sheep. Second, the decrease in lactose secretion by 5% in HS goats (Hamzaoui et al., 2012) may spare some glucose in blood as 80 to 85% of glucose in blood is used by the mammary gland for lactose synthesis in goats (Sano et al., 1985) and cows (Bickerstaffe et al. 1974). Finally, there is probably some degree of body muscle degradation under HS (Wheelock et al., 2010) and the resultant amino acids could be used for gluconeogenesis.

Figure 4.5. Insulin response to the glucose tolerance test of dairy goats under thermal neutral (TN; n = 4) or heat stress (HS; n = 4) conditions. Discontinued arrow indicates time of glucose injection. Values are means with SE indicated by vertical bars. * indicates a difference at $P< 0.05$ between TN and HS treatments.



4.4.3 Behavioral indices

For the behavioral indices in the current study, the day was divided into 2 portions: daylight from 900 to 2100 during which HS goats were at 37ºC with artificial light, and night from 2100 to 900 during which HS goats were at 30ºC in dark. The TN goats were at 15-20ºC throughout the day with similar light regimen. Haley et al. (2000) and Fregonesi and Leaver (2001) reported that the time spent lying down and the duration of individual bouts are sensitive measures of stall comfort and animal welfare. Therefore, information about duration and frequency of different activities could reflect the comfort of goats under HS conditions.

Obtained behavioral indices in TN and HS goats are shown in Table 4.3 and Figures 4.6 and 4.7. During the daylight TN goats remained standing (for eating, drinking or idling) for longer time (68%) than HS goats (39%). During the night, TN and HS goats spent shorter time standing (26%) compared to the daylight with no difference between groups (Figure 4.6). Changing the position (from lying down to standing and vice versa) during daylight and night was much greater ($P < 0.01$) in HS (18.9 times as daily average) than TN goats (7.1 times as daily average). The increment in the position changing frequency indicates that HS goats were uncomfortable and had extra-movements. These extra-movements jointly with muscle movements for panting could increase the maintenance requirements for HS animals.

Total daily eating bouts (41.7) were similar between groups and greater ($P < 0.001$) during the daylight (29.1) than during the night (12.6). However, HS goats doubled their number of eating bouts during the night when temperature decreased from 37 to 30ºC. Throughout the day, TN and HS had similar number of visits to the feed trough, but the duration of each meal was shorter ($P < 0.01$) in HS (5.7 min) than in TN (8.7 min) goats (Table 4.3).This could explain the reduction in DMI observed when goats were in HS. On the other hand, the greater water consumption by HS could be explained by the greater number of drinking bouts in HS (30.4 bouts) than in TN (12.8 bouts) with similar time devoted to each bout in both groups (0.48 min on average). Cook et al. (2007) showed that time spent drinking by dairy cows increased from 0.3 to 0.5 h/d when THI increased from 56 to 74.

Percentages of time devoted to different activities (eating, drinking or idling) while standing are shown in Figure 4.7. During the daylight and in accordance with changes in DMI and water consumption, HS goats spent greater percentage of time drinking and idling, but lower portion of time eating than TN goats. During the night, HS goats reduced the portion of time devoted to idling and spent a greater portion of time eating when ambient temperature decreased.

Table 4.3. Behavior indices during the daylight (12 h) and night (12 h) of dairy goats under thermal neutral (TN; n = 8) and heat stress (HS; n = 8) conditions. Values are least squares means and SE of the mean (SEM). Goats in TN were kept at 15-20°C throughout the day, whereas HS goats were at 37°C during the daylight and at 30°C during the night.

Items	TN		HS			Effect (*P*-value)		
	Day	Night	Day	Night	SEM	Treatment	Daytime	T x D[1]
Position change[2], n	7.6	6.6	22.6	15.1	1.8	0.003	0.018	0.060
Standing time, min	486	177	278	196	14	0.004	0.001	0.001
Lying time, min	234	543	442	524	17	0.004	0.001	0.001
Lying time average[3], min	35.0	92.3	22.3	27.7	7.7	0.002	0.001	0.010
Eating bouts, n	32.1	8.8	26.0	16.4	2.3	0.817	0.001	0.002
Eating time, min	273	71	105	97	9	0.001	0.001	0.001
Eating time/meal, min	8.75	8.65	4.76	6.58	3.0	0.006	0.284	0.233
Drinking bouts, n	10.5	2.3	21.6	8.8	2.2	0.013	0.002	0.408
Drinking time, min	5.8	1.3	8.4	4.4	0.94	0.048	0.017	0.875
Drinking time/bout, min	0.54	0.43	0.53	0.40	0.09	0.175	0.828	0.942

[1] Interactions of treatment (T) and daytime (D).[2] Counted as the change from standing to lying down and vice versa.[3] Calculated as total lying time divided by the times of position changing.

Although our goats were in metabolic cages, time spent lying down by TN goats (13 h/24 h) was similar to the 12-13 h reported for healthy dairy cows housed in a free-stall barn (Cook et al., 2005; Drissler et al., 2005). On the other hand, HS increased the total lying down duration by 3 h (16 h /24 h), but the duration of each lying down action was shorter in HS (22 and 28 min for daylight and night, respectively) than in TN (35 and 92 min during daylight and night, respectively) due to the greater position changing frequency. In contrast to our results, the duration of lying behavior decreased with increasing THI in dairy cows housed in free-stalls (Shultz, 1984; Overton et al. 2002; Zahner et al., 2004, Cook et al., 2007). The discrepancy could be due to specie differences and the fact that goats in the current study were kept in metabolic cages, while cows in the mentioned studies were in free-stalls and able to move looking for cooler zones (close to sprinklers, fans, etc.).

Figure 4.6. Percentage of time remaining standing or lying down during the daylight (12 h) and night (12 h) in dairy goats under thermal neutral (TN; n = 8) or heat stress (HS; n = 8) conditions.

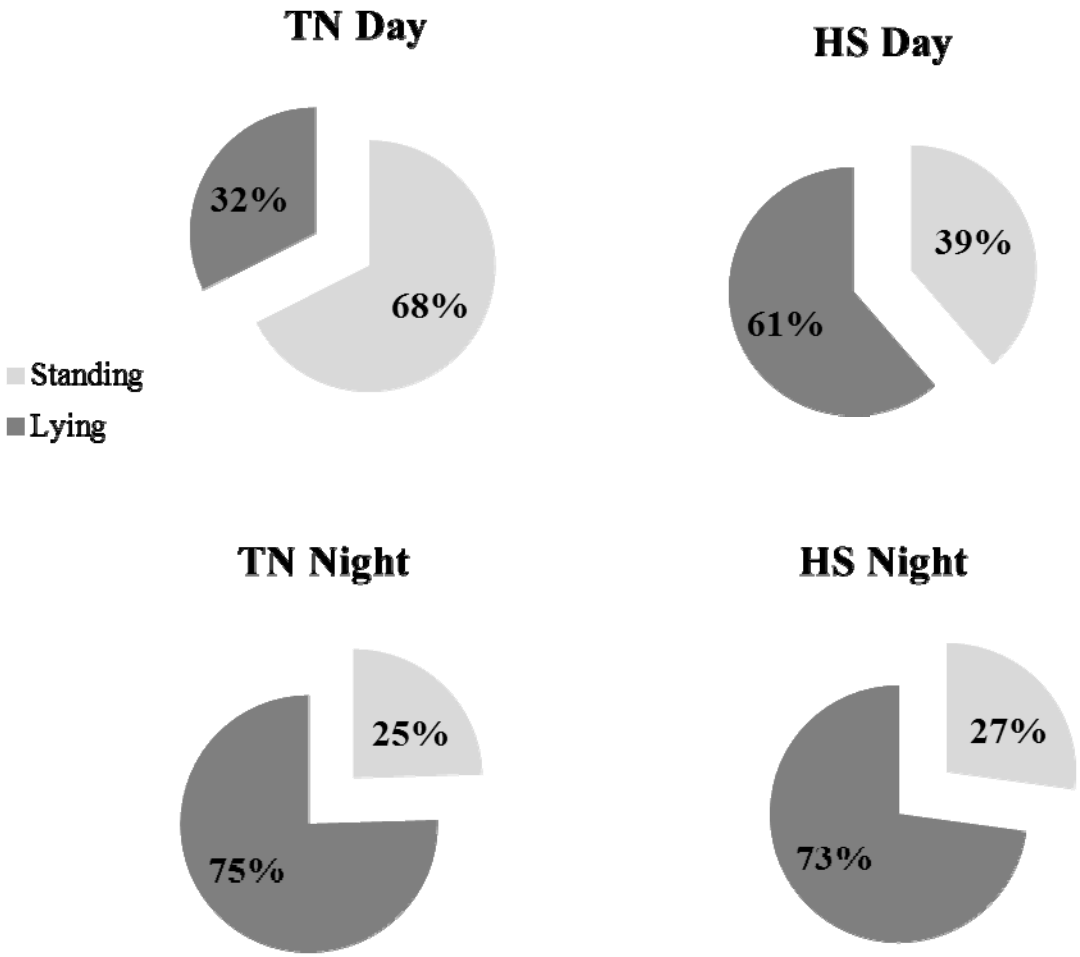
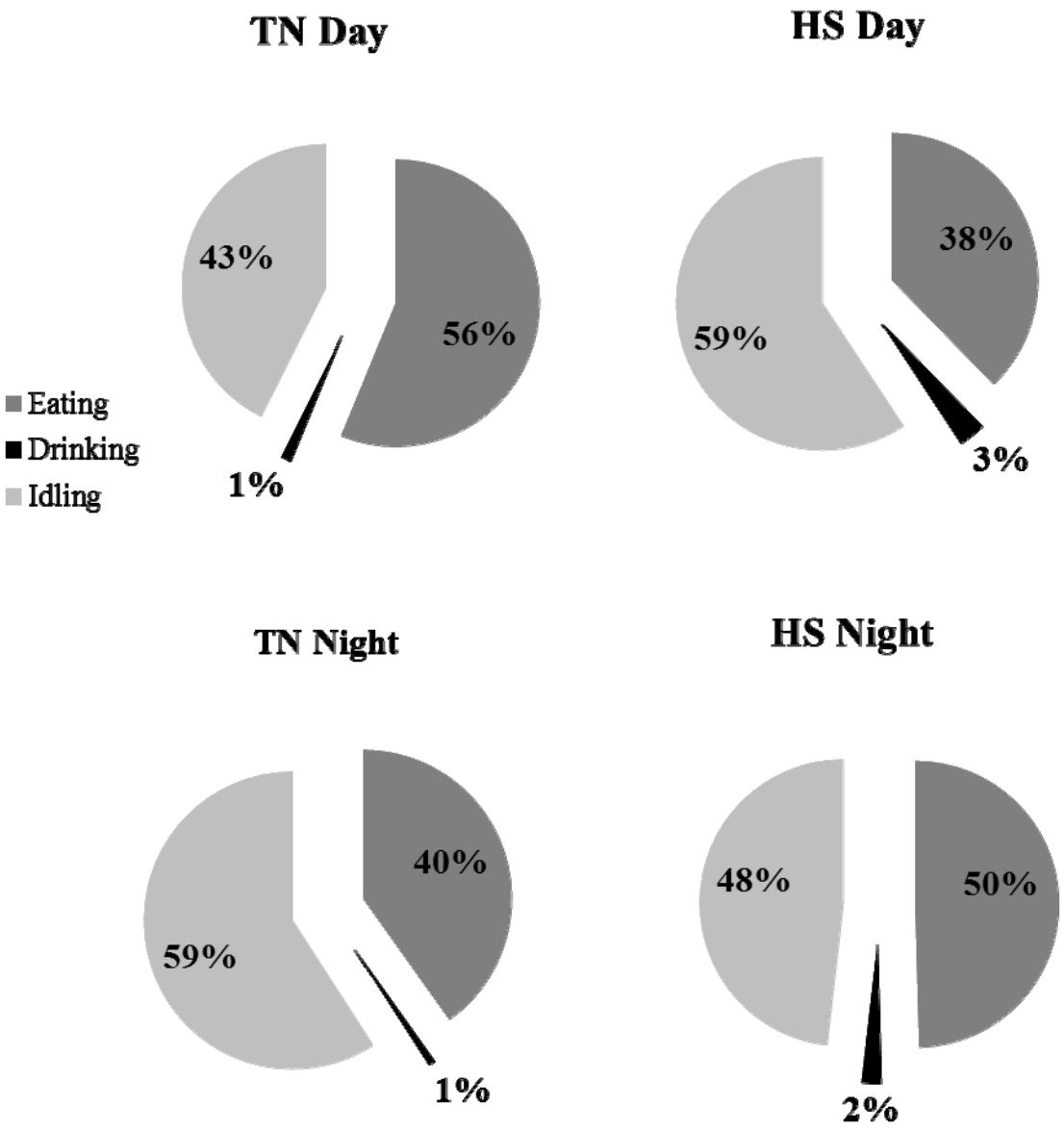


Figure 4.7. Portions of time when standing devoted to eating, drinking or idling during the daylight (12 h) and night (12 h) in dairy goats under thermal neutral (TN; n = 8) or heat stress (HS; n = 8) conditions. These portions were calculated for the 3 activities while goats were standing.



4.5 CONCLUSIONS

The negative impact of heat stress on milk yield and milk components is conditioned by the stage of lactation, being greater in goats at early- mid-lactation than in later stages. Heat-stressed goats had the same response to lipogenic hormones, but the adipose tissue was less sensitive to lipolytic signals, which explains the lack of fat mobilization under heat stress conditions. It seems that the pancreas of heat stress goats was less sensitive, secreting lower insulin amounts in response to glucose administration. This finding might be a mechanism by which goats keep blood glucose levels in high ambient temperatures, even with reduced feed intake. Heat stress had no effect on eating bouts, but the reduced feed intake observed during heat stress was due to the shorter time of each eating bout. The increment in water consumption in the hot ambient was due the elevated number of drinking bouts rather than the duration of drinking bouts.

CHAPTER 5

Increasing milk fat and improving fatty acid profile under heat stress in dairy goats by supplementation with soybean oil

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Increasing milk fat and improving fatty acid profile under heat stress in dairy goats by supplementation with soybean oil

5.1 ABSTRACT

In a previous work we observed that heat-stressed goats suffered reductions in milk yield, milk fat, and milk protein. Supplementation with soybean oil (SBO) may be a useful way to enhance milk quality. Eight multiparous Murciano-Granadina dairy goats (42.8 ± 1.3 kg BW; 99 ± 1 DIM) kept in metabolic cages were used in a replicated 4 × 4 Latin square design with 4 periods; 21 d each (14 d adaptation, 5 d for measurements and 2 d transition between periods). Goats were allocated to one of 4 treatments in a 2 x 2 factorial arrangement. Factors were no oil (C) or 4% of soybean oil (S), and thermal neutral (TN; 15 to 20°C) or heat stress (HS; 12 h/d at 37°C and 12 h/d at 30°C) conditions. This resulted in 4 treatment combinations: TN-C, TN-S, HS-C, and HS-S. The humidity was maintained at 40 ± 5%. Feed intake, water consumption, BW, milk yield, milk composition, milk fatty acid profile, digestibility, rectal temperature (RT), respiration rate (RR) and blood indicators were measured. Compared to TN, HS goats had lower ($P < 0.05$) feed intake, BW, N balance, milk yield, milk protein and milk lactose content. Moreover, goats under HS had 5 to 9 points greater ($P < 0.05$) digestibility coefficients than TN goats. From the point of view of human health, HS improved milk fatty acid profile by decreasing saturated fatty acids (SFA) and increasing monounsaturated fatty acids (MUFA), with no effect on milk fat content. The SBO increased ($P < 0.05$) on average blood NEFA by 50%, milk fat by 30%, and conjugated linoleic acid (CLA) by 360%. The response to SBO waswith the same magnitudein TN and HS conditions. In conclusion, feeding SBO to heat-stressed dairy goats was a useful way to increase milk fat, CLA, without any negative effects on intake, milk yield, or milk protein content.

5.2 INTRODUCTION

Typically, milk production in dairy animals is negatively affected by heat stress (HS) because DMI is reduced and a portion of consumed energy is directed to maintain homeothermy. Consequently, the availability of energy for lactation is less, and lower amount of milk is produced with lower contents of fat and protein. Staples and Thatcher (2011) examined the relationship between milk composition and environmental temperature and found that as temperatures increased from 9.4 to 36.1ºC, milk fat and protein contents dropped by 14 and 13%, respectively. Similarly, heat-stressed dairy goats produced -9% of milk that contained -12% fat and -13% protein compared to goats in thermal-neutral (TN) conditions (Hamzaoui et al., 2013a; Salama et al., 2014).

Numerous studies have been carried to evaluate the effects of fat supplementation under hot conditions. Feeding fat is associated with reduced metabolic heat production per unit of energy fed (Baldwin, et al., 1980), and compared to starch and fiber, fat has a much lower heat increment in the rumen (Van Soest, 1982). Therefore, fats feeding under HS conditions could be beneficial. However, reports on the fat supplementation under hot conditions were inconsistent (Moody et al., 1967, 1971; Saunders et al., 1990; Knapp and Grummer, 1991; Drackley et al., 2003). Differences between studies carried out on ruminants are probably due to the fact that excess ruminally active fat in the diet may impair ruminal fermentation (Van Nevel and Demeyer, 1988). Moreover, animal fats and vegetable oils may reduce the palatability of the ration. Bernabucci (2012) suggested that the use of protected fat is advisable to avoid the deleterious effect of fat excess on rumen microflora.

Bouattour et al. (2008) indicated that the addition of SBO to the ration of non HS-dairy goats increased significantly fat content and CLA in milk. In the literature, no studies are available to evaluate the effect of SBO in summer (i.e. HS conditions) or in non HS conditions in dairy goats. The current study evaluates the response to SBO in HS and TN conditions simultaneously using the same goats in the same lactation stage. This study was conducted to evaluate the effect of supplementation with SBO on milk production and fatty acid profile in HS dairy goats in mid-lactation.

5.3 MATERIALS AND METHODS

5.3.1 Animal and Management Conditions

Animal care conditions and management practices agreed with the procedures stated by the Ethical Committee of Animal and Human Experimentation of the Universitat Autònoma de Barcelona (Bellaterra, Spain; CEEAH reference 09/771) and the codes of recommendations for the welfare of livestock of the Ministry of Agriculture, Food and Environment of Spain (Madrid).

Eight multiparous Murciano-granadina dairy goats (99 ± 1 DIM, 2.00 ± 0.04 L/d of milk yield; 42.8 ± 1.3 kg BW) with healthy and symmetrical udders were used from the herd of the experimental farm of the Universitat Autonoma de Barcelona. Goats were kept in metabolic cages and were used in a replicated 4 × 4 Latin square design with 4 periods; 21 d each (14 d adaptation, 5 d for measurements and 2 d transition between periods). When goats were switched from TN to HS conditions, a transition period of 2 d was allowed (1 d at 25°C, 1 d at 30°C), but the change from HS to TN was abrupt. Body weight of each goat was recorded at the start and the end of each period.

Goats were allocated to one of 4 treatments in a 2 × 2 factorial arrangement. Factors were no oil (C) or 4% of soybean oil (S), and thermal neutral (TN; 15 to 20°C) or heat stress (HS; 12 h/d at 37°C and 12 h/d at 30°C) conditions. This resulted in 4 treatment combinations: TN-C, TN-S, HS-C, and HS-S. The humidity was maintained at 40 ± 5%. Data of environmental temperature and humidity were recorded every 10 min throughout the experiment by a data logger (Opus 10, Lufft, Fellbach, Germany) and THI values were calculated according to NRC (1971).

The daily ration for all goats consisted of TMR that contained: alfalfa hay 60.4%, ground barley grain 15%, beet pulp 9.1%, ground corn grain 7.5%, soybean meal 3%, sunflower meal 3%, molasses 1%, salt 0.6%, sodium bicarbonate 0.2%, and CVM for goats 0.2%. Chemical composition and nutritive value of the ration are shown in Table 5.1. Mineral and vitamin blocks were freely available for each goat. The soybean oil was daily added to the ration by replacing a similar amount of barley grains.

Table 5.1. Chemical composition and nutritive value (DM basis) of the total mixed ration used for dairy goats.

Item	Total mixed ration
Component, %	
Dry matter	89.92
Organic matter	87.34
Crude protein	17.20
Neutral detergent fiber	34.36
Acid detergent fiber	21.79
Nutritive value[1]	
UEm,[2] /kg	0.71
UFL,[3] /kg	0.82
NE_L, Mcal/kg	1.40
PDIE,[4] g/kg	100
PDIN,[5] g/kg	116
PDIA,[6] g/kg	50
Ca, g/kg	10.55
P, g/kg	2.86

[1]Calculated according to Institut National de la Recherche Agronomique (INRA, 2007). [2] Fill units for sheep (1 UEm = 1 kg of reference grass DM). [3] Feed units for lactation (1 UFL = 1.7 Mcal of NE_L). [4] Protein digested in the small intestine supplied by microbial protein from rumen-fermented OM. [5] Protein digested in the small intestine supplied by microbial protein from RDP. [6] Protein digested in the small intestine supplied by RUP.

Goats were milked once daily (0800 h) with a portable milking machine (Westfalia-separator Ibérica, Granollers, Spain) with recorded jars (3 L ± 5%). Milking was conducted at a vacuum pressure of 42 kPa, a pulsation rate of 90 pulses/min, and a pulsation ratio of 66%. The milking routine included cluster attachment without udder preparation or teat cleaning, machine milking, machine stripping before cluster removal, and teat dipping in an iodine solution (P3-ioshield, Ecolab Hispano-Portuguesa, Barcelona, Spain).

5.3.2 Measurements, Sample Collection, and Analyses

5.3.2.1 Rectal Temperature and Respiration Rate

Rectal temperatures and respiration rates were daily recorded at 0800, 1200, and 1700 h. The rectal temperature was measured by a digital clinical thermometer (Model ICO

Technology "mini color" Barcelona, Spain (32 to 43.9 ± 0.1ºC). The respiration rate was measured by counting the inhalations and exhalations for 60 s with the aid of a chronometer.

5.3.2.2 Feed Intake and Water Consumption

Feed intake and water consumption (accuracy: ± 20 g) were recorded daily throughout the experiment. Trays with saw dust were put below the drinking troughs and weighted twice daily to take into account water wastes. Feed samples were collected daily during measurement days of each period and were ground through a 1-mm stainless steel screen, and then analyzed for DM, ADF, NDF, and ash content according to analytical standard methods (AOAC International, 2003). The Dumas method (AOAC International, 2003) with a Leco analyzer (Leco Corp., St. Joseph, MI) was used for N determinations and CP was calculated as percentage of N × 6.25.

5.3.2.3 Milk Yield and Milk Composition

Milk yield of individual goats was recorded daily throughout the experiment and milk composition was evaluated for each period. Milk samples of approximately 50 mL were collected in2 consecutive days during the measurement days and preserved with an antimicrobial tablet (Bronopol, Broad Spectrum Microtabs II, D&F Control Systems Inc., San Ramon, CA) at 4°C until analysis. Milk samples were analyzed with a near-infrared spectrometer (Foss NIRSystems 5000, Foss, Hillerød, Denmark) for contents of fat, protein (N × 6.38), and lactose.A milk sample of approximately 50 mL was collected individually in each period, fat was obtained by centrifugation at 6000 × *g* for 30 min, and frozen at -80°C until the analysis of fatty acid profile using gas chromatography method as described by Bouattour et al. (2008).

5.3.2.4 Blood Measures

Blood samples were i.v. collected once from each goat in each period into vacutainers (Venoject, Leuven, Belgium) before the morning feeding. Plasma was obtained by centrifugation of whole blood for 15 min at 1500 ×*g*, and stored at -20°C for the analysis of NEFA and β-hydroxybutyrate acid (BHBA). The NEFA were determined by the colorimetric enzymatic test ACS-ACOD method using a commercial kit (Wako Chemicals, Neuss, Germany). The BHBA was determined by kinetic enzymatic method using commercial kit (RANBUT, Randox®, UK). Moreover, blood samples were collected by insulin syringes. A

single drop of blood was applied to disposable cartridges containing biochemical and silicon chip technology (i-STAT EC8+, Abbott Point of Care Inc., Princeton, NJ). Then, the cartridge was inserted into an i-STAT handheld analyzer, and the results of glucose, urea, Cl, Na, K, total CO_2 concentration, anion gap, hematocrit, hemoglobin, pH, partial pressure of CO_2, HCO_3^-, and base excess were obtained.

5.3.2.5 Digestibility Coefficients

During the 5 measurement days of each period, feed orts were daily collected, weighed, and composted for the analysis. Feces of each goat were daily collected and 10% of fresh feces were dried at 60°C for 48 h. Then a composted sample for each goat was stored at room temperature until analysis. Orts and feces samples were ground through a 1 mm stainless steel screen and then analyzed for DM, CP, CF, ADF, NDF and ash.

5.3.3 Statistical Analyses

Data were analyzed by the PROC MIXED for repeated measurements of SAS (v. 9.1.3, SAS Institute Inc., Cary, NC). The statistical mixed model contained the fixed effects of the treatment (TN-C, TN-S, HS-C and HS-S) and period; the random effect of the animal; the interactions treatment × period; and the residual error. The model took into account the possible carryover effects of previous HS periods through the treatment × period interaction. Data of performances (i.e., intake, water, and milk yield) and physiological indicators (i.e., rectal temperature and respiratory rate) were analyzed on a daily basis.

Data of digestibility and N balance were analyzed using PROC GLM of SAS. The model contained the effect of treatment and period; the interaction between treatment and period; and the residual error.

5.4 RESULTS AND DISCUSSION

5.4.1 Rectal Temperature (RT) and Respiration Rate (RR)

Rectal temperatures and respiration rates data at 0800, 1200 and 1700 h are shown in Table 5.2. The HS goats showed a greater ($P < 0.01$) RT and RR than TN goats. Similarly, HS increased RT and RR in dairy cows (Rhoads et al., 2009; Shwartz et al., 2009) and goats (Hamzaoui et al., 2013a). The increment in RR under HS conditions is a known mechanism for dissipating heat load by evaporation.

The supplementation with SBO had no effect on RT and RR in our goats. O'Kelly (1987) fed diets containing 9.2% fat during hot weather to both Brahman cross and British crossbred steers and reported lower body temperatures (0.3 to 0.4◦C lower) compared with steers fed diets containing 2.5% fat, which suggests less heat production in those steers. In contrast, Gaughan and Mader (2009) reported an increase of body temperature and respiration rate in finishing steers exposed to hot and fed 5% of soybean oil. Furthermore, (Chan et al., 1997) reported that feeding diets containing 4.6% or 7.4% fat and housing in shade or shade plus an evaporatively cooled environment did not improve milk or fat-corrected milk yields and had no effects on rectal temperatures.

5.4.2 Feed Intake, Water Consumption and Body Weight Change

On average, DMI decreased ($P < 0.001$) by 35% and 41% due to HS in C and S goats, respectively (Table 5.2). Goats in the current experiment were in mid lactation, and DMI loses were greater than those previously reported (-21%) in late lactating goats under HS (Hamzaoui et al., 2013a). Reducing feed intake is a way to decrease heat production in warm environments because the heat increment of feeding, especially in ruminants, is an important source of heat production (Appleman and Delouche, 1958; Kadzere et al., 2002; Salama et al., 2014).

The HS goats lost 115 g/d of BW due to the reduction in DMI, whereas TN goats gained 162 g/d. The HS goats were in negative energy balance (-0.22 Mcal/d), whereas TN goats had a positive energy balance (+0.95 Mcal/d), which could explain the changes observed in BW. Moreover, a portion of the changes in BW of TN and HS goats included the inevitable variations in the digestive tract content, which were unknown in our data.

Supplementation with SBO did not affect the DMI, which agrees with the results obtained when vegetable oils (sunflower, linseed, or soybean) were supplemented (2 to 5%) to dairy goats (Bouattour et al., 2008; Martinez Marin et al., 2012) and cows (Huang et al., 2008; O'Donnell-Megaro et al., 2012).

The HS goats increased ($P < 0.001$) water consumption of HS goats by 83% on average compared to TN goats independently of SBO supplementation (Table 5.2). Increased water intake was mainly used under HS conditions for boosting heat loss by evaporation from the skin (sweating) and by respiration (panting) as previously observed by Hamzaoui et al. (2013a).

Table 5.2. Lactational performances of Murciano-Granadina dairy goats under thermal neutral (TN) and heat stress (HS) conditions. In each ambient, goats were not supplemented (C) or supplemented with 4% soybean oil (S). Values are LSM and SE of the means (SEM).

	TN		HS					
Item	C	S	C	S	SEM	Treatment	Ration	T x R[1]
Goats, n	8	8	8	8	-	-	-	-
Rectal Temperature, ºC								
0800h	38.5	38.5	39.1	39.4	0.08	0.001	0.091	0.115
1200h	38.7	38.7	39.7	39.8	0.07	0.001	0.248	0.370
1700h	38.7	38.8	39.9	39.9	0.09	0.001	0.461	0.884
Respiration Rate, breaths/min								
0800h	27.0	27.0	69.0	74.0	3.47	0.001	0.399	0.415
1200h	38.5	38.7	131.0	133.9	5.91	0.001	0.798	0.824
1700h	37.1	37.8	130.1	134.4	6.00	0.001	0.668	0.786
Intake								
DM, kg/d	2.26	2.26	1.47	1.34	0.09	0.001	0.490	0.470
Water, L/d	6.1	6.3	10.6	12.1	1.04	0.001	0.310	0.480
BW, Kg	48.58	42.23	39.84	38.11	1.82	0.001	0.350	0.214
BW variation, Kg	3.49	2.65	-2.08	-2.28	0.97	0.001	0.597	0.745
Milk, L/d	1.88	1.99	1.79	1.75	0.11	0.013	0.606	0.230
3.5% FCM[2], L/d	2.17	2.31	1.86	2.1	0.13	0.004	0.035	0.560
Milk composition, %								
Fat	3.98	5.07	3.64	4.85	0.20	0.179	0.001	0.781
Protein	3.40	3.40	2.85	2.96	0.10	0.001	0.561	0.570
Lactose	4.51	4.66	4.3	4.43	0.07	0.004	0.057	0.858
Digestibility, %								
DM	67.8	68.5	74.0	72.6	1.4	0.001	0.778	0.455
OM	68. 9	69.6	75.1	73.9	1.3	0.001	0.850	0.469
CP	73.4	74. 7	78.8	78.6	1.3	0.001	0.654	0.559
NDF	50.5	50.2	58.1	56.6	2.4	0.007	0.708	0.804
ADF	43.5	43.6	52.5	52.8	2.9	0.004	0.941	0.989
N Balance, g	21.8	20.2	13.7	15.5	2.3	0.009	0.951	0.454

[1]Interaction of treatment (T) × Ration (R).[2] Fat corrected milk at 3.5%; FCM = L × [0.432 + 0.162 × (fat %)], being L liters of milk yield.

5.4.3 Milk yield and Milk Composition

As shown in Table 5.2, HS goats produced lower ($P < 0.01$) milk yield and FCM than TN goats. Late lactating goats of the same breed suffered no losses in milk yield under HS conditions (Hamzaoui et al., 2013a). It seems that the response of milk yield to HS varies according to lactation stage, with goats at earlier stages (e.g. current study and Chapter 5) suffering greater losses. The supplementation with SBO did not modify milk yield, which is similar to what has been observed in dairy goats (Bouattour et al., 2008) and cows (Huang et al., 2008; Jacobs et al., 2011; O'Donnell-Megaro et al., 2012).

When SBO was fed to TN and HS goats, milk fat content increased by 27 and 33%, respectively (Table 5.2). Chilliard et al. (2003) and Bouattour et al. (2008) reported increased milk fat content when goats were supplemented with SBO. In contrast, Huang et al. (2008) reported a decrease in milk fat content of dairy cows supplied by 5% of SBO. Other studies reported no change in milk fat content due to SBO in dairy cows (Jacobs et al., 2011) or ewes (Gómez-Cortés et al., 2008). These contradictive results could be due to differences in specie, breed, physiological state, roughage source, and roughage: concentrate ratio in the diet (Chilliard et al., 2003).

Compared to the TN conditions, HS treatment decreased ($P < 0.001$) protein content in milk by 15% as previously observed in dairy goats (Hamzaoui et al., 2012; 2013a). This decrease in milk protein could be explained by the increased sweat secretion that contains protein and urea (Joshi et al., 1968) together with decreased protein intake under HS, which might have limited the availability of amino acids for milk protein synthesis (Salama et al., 2014). Moreover, lactose content in milk was decreased by the HS ($P < 0.01$) in agreement with losses in milk yield.

Addition of SBO under TN or HS conditions increased milk fat without any negative effect on milk protein or lactose contents (Table 5.2). This result agrees with what obtained by others in non heat-stressed dairy goats supplemented with SBO (Chilliard et al., 2003; Bouattour et al., 2008) or other vegetable oils (Martínez-Marín et al., 2012). In addition, Huang et al. (2008) and Jacobs et al. (2011) did not report any changes in protein or lactose contents in dairy cows supplemented with SBO.

5.4.4 Milk Fatty acids (FA) profile

Data of milk FA profile as affected by HS and SBO supplementation are shown in Table 5.3. The SBO and HS had opposite effect on milk fat content; milk fat was increased ($P < 0.001$) by SBO and numerically decreased ($P = 0.179$) by HS. Nevertheless, HS and SBO supplementation generally had a similar effect ($P < 0.001$) on milk FA profile, as both of them decreased short- (<12 C) and medium-chain (C12+C16) FA, and increased long chain (>C16) FA. Moreover, both HS and SBO supplementation decreased ($P < 0.001$) the concentrations of saturated FA and increased ($P < 0.001$) monounsaturated FA concentrations without any effect on the polyunsaturated FA.

The increase in long chain FA when SBO was supplemented could be due to the increment in blood NEFA levels by more than 50% on average (see later). These NEFA are taken up by the mammary gland and used for milk fat synthesis. The increase in long chain FA has an inhibitor effect on de novo FA synthesis in the mammary gland (Chilliard et al., 2003), which resulted in lower concentrations of short- and medium-chain FA in SBO goats. The situation in case of HS is totally different as no increase in blood NEFA values was observed (see later) to justify the increase in long chain FA. The de novo synthesis of FA decreased by HS, which resulted in an increment in the proportion of long chain FA in milk.

Taking into account data of milk fat content (Table 5.2) and FA (Table 5.3), fat yields of TN and HS goats were 74.8 g/d (27.9 g < C16; 29.3 g C16:0 + C16:1; 17.1 g > C16) and 65.2 g/d (22.2 g < C16; 20.3 g C16:0 + C16:1; 22.2 g > C16), respectively. Thus, due to HS the production of totally (<C16) or partially (C16 + C16:1) de novo FA was reduced (-14.8 g/d), whereas FA extracted from blood increased by only +5.1 g/d. The reduction in de novo FA synthesis by HS decreases the needs of energy and could spare mammary glucose. Moreover, the decrease in mammary lactose synthesis under HS spares more glucose, which might partially explain why HS goats had similar blood glucose to TN goat even with lower DMI (Hamzaoui et al., 2013a; Salama et al., 2014). Changes in rumen biohydrogenation pathways could not be excluded as a source of variation in milk FA under HS conditions. By the aid of rumen pH and temperature sensors, we observed that HS-goats had lower rumen pH and greater temperature, even when DMI was the same as for TN goats (Salama et al., 2014).

Table 5.3. Fatty acids profile (% of total FA) of Murciano-Granadina dairy goats under thermal neutral (TN) and heat stress (HS) conditions. In each ambient, goats were not supplemented (C) or supplemented with 4% soybean oil (S).Values are LSM and SE of the means (SEM).

	TN		HS			Effect (*P*<)		
Item	C	S	C	S	SEM	Treatment	Ration	T x R[1]
Goats, n	8	8	8	8	-	-	-	-
C6:0	2.02	2.17	1.94	2.14	0.11	0.462	0.042	0.771
C8:0	2.61	2.81	2.68	2.53	0.17	0.451	0.856	0.224
C10:0	11.45	9.79	10.70	7.73	0.60	0.005	0.001	0.140
C12:0	6.91	4.24	5.74	2.80	0.51	0.005	0.001	0.724
C14:0	12.94	9.71	11.94	7.50	0.69	0.011	0.001	0.279
C14:1	0.268	0.153	0.148	0.083	0.05	0.006	0.008	0.399
C15:0	0.935	0.683	0.793	0.578	0.05	0.003	0.001	0.589
C16:0	38.23	25.68	30.59	22.11	1.81	0.001	0.001	0.154
C16:1	1.00	0.60	0.63	0.44	0.19	0.028	0.018	0.335
C17:0	0.55	0.43	0.75	0.46	0.06	0.008	0.001	0.033
C18:0	4.87	11.8	9.07	17.29	1.73	0.003	0.001	0.622
C18:1n9t	0.14	0.56	0.15	0.65	0.04	0.095	0.001	0.225
C18:1n11t (TVA[2])	0.68	4.76	0.71	5.68	1.49	0.614	0.001	0.640
C18:1n9c	12.58	19.59	18.84	23.00	1.29	0.001	0.001	0.183
C18:1n11c	0.37	0.78	0.54	0.97	0.05	0.001	0.001	0.775
C18:2n6t	0.18	0.42	0.17	0.43	0.04	0.968	0.001	0.840
C18:2n6c	2.48	2.55	2.9	2.67	0.17	0.045	0.529	0.226
C20:0	0.15	0.22	0.18	0.26	0.01	0.023	0.001	0.536
C18:3n3+C20:1	0.69	0.55	0.72	0.5	0.05	0.733	0.001	0.315
c9 t11 (CLA[3])	0.47	2.17	0.37	1.95	0.62	0.685	0.001	0.875
C22:0	0.05	0.09	0.05	0.11	0.02	0.719	0.004	0.591
C20:4n6	0.15	0.10	0.23	0.13	0.02	0.003	0.001	0.840
SFA	80.95	67.76	74.52	63.5	1.32	0.001	0.001	0.381
MUFA	15.03	26.46	21.01	30.82	1.27	0.001	0.001	0.399
PUFA	3.49	3.62	4.01	3.72	0.23	0.060	0.612	0.198
MUFA+PUFA	18.52	30.07	25.01	34.54	1.32	0.001	0.001	0.326
<C16[4]	37.36	29.70	34.04	23.36	1.00	0.001	0.001	0.090

C16+C16:1[4]	39.23	26.28	31.21	22.56	1.81	0.001	0.001	0.140
>C16[4]	22.87	43.6	34.00	53.64	2.06	0.001	0.001	0.703
n-3	0.72	0.50	0.69	0.55	0.05	0.655	0.001	0.216
n-6	2.80	3.07	3.29	3.22	0.19	0.024	0.428	0.192
n-6:n-3	4.05	5.66	4.62	6.44	0.22	0.001	0.001	0.537
Elongase[6]	30.87	54.28	47.17	63.93	3.07	0.001	0.001	0.169
TVA/CLA	1.61	2.26	1.93	3.01	0.28	0.027	0.002	0.337
AI[5]	5.26	2.29	3.40	1.60	0.29	0.001	0.001	0.015
Δ^9-Desaturase index[7]								
C14	0.020	0.015	0.013	0.010	0.006	0.096	0.300	0.724
C16	0.025	0.023	0.02	0.018	0.003	0.049	0.295	1.000
CLA[3]	0.40	0.31	0.34	0.25	0.040	0.039	0.004	0.885

[1] Interaction of treatment (T) × Ration (R).[2] Trans vaccenic acid.[3] Conjugated linoleic acid, [4]<C16 de novo synthesis, >C16 taken up by the gland, C16 + C16:1 de novo and preformed.[5] Atherogenicity index calculated according to Ulbricht and Southgate (1991) as: (12:0 + 4 × 14:0 + 16:0)/(MUFA + PUFA).[6] Elongation of C16 to C18 calculated as (C18 + C18:1)/(C16 + C16:1 + C18 + C18:1) ×100. [7] Calculated for each pair of FA according to Kelsey et al. (2003) as (product of Δ^9-desaturase)/(product of Δ^9-desaturase + substrate of Δ^9-desaturase); e.g., C14:C14:1/(C14:1 + C14:0).

The SBO supplementation, but not HS, dramatically increased CLA content in milk of dairy goats (Table 5.3) as a consequence of the increment in TVA. This result is similar to what previously reported in dairy goats (Bouattour et al., 2008), ewes (Gómez-Cortés et al., 2008) and cows (Palmquist et al., 2005 and Bu et al., 2007).The effect of SBO on TVA and CLA contents was similar in TN and HS conditions (+650 and +395% increments in TVA and CLA concentrations, respectively on average). There is a strong positive correlation between CLA and TVA levels in milk of dairy goats (Chilliard et al., 2003), ewes (Cabiddu et al., 2005) and cows (Griinari et al., 2006).

From the point of view of human health, HS and SBO reduced milk atherogenicity index by -32 and -54% (Table 5.3). When the effects of HS and SBO were jointed (i.e. HS-S goats), the atherogenicity index was dramatically reduced by -70% compared to the control (i.e. TN-C goats). Moreover, milk of HS and SBO goats contained lower values of saturated FA and greater contents of mono-unsaturated FA. The mono-unsaturated FA are advantageous as they increase the concentration of high density lipoproteins that prevent cholesterol from accumulation on blood vessel walls and transport it to the liver (Markiewicz-Kęszycka et al., 2013).

We should keep in mind that although HS resulted in healthier milk FA profile, it negatively affected milk protein, and could impair milk coagulation properties and reduce cheese yield in dairy goats (Abdel-Gawad et al., 2012; Salama et al., 2014).

5.4.5 Digestibility and Nitrogen Balance

Data of digestibly coefficients (DM, CP, NDF and ADF) and N balance are shown in Table 5.2. The HS increased ($P < 0.01$) all digestibilities by 5 to 9 points. The ADF digestibility improvement was the highest (+9 points) followed by NDF digestibility (+7 points). This increment in digestibility is greater than the observed in the same goat breed at late lactation (Hamzaoui et al., 2013a). Similarly, greater digestibility by HS has been observed in male goats (Hirayama et al., 2004), dairy cows (McDowell et al., 1969), and heifers (Bernabucci et al., 1999). The increased digestibility under HS conditions might be partially due to the reduction of feed intake. Another reason for the enhanced digestibility under HS conditions could be a depressed passage rate of the solid phase of digesta as proposed by Bernabucci et al. (1999) and Salama et al. (2014). Due to HS, N intake and N balance decreased by 38 and 30%, respectively. In contrast, we observed in a previous study that HS had no effect on N balance in late lactation dairy goats, even with less N intake (Hamzaoui et al., 2013a). Lower retained N could partially explain the reduction in milk protein in HS goats.

The SBO supplementation did not affect the digestibility or N balance of dairy goats ($P > 0.05$). Adding vegetable oils seems advantageous by enhancingmilk quality with neutral effect on digestibility parameters.

5.4.6 Blood indicators

Data of blood indicators in TN and HS goats supplemented or not with SBO are shown in Table 5.4. The HS decreased ($P < 0.05$) or tended ($P < 0.10$) to decrease Na, K, tCO_2, pCO_2, HCO_3^-, base excess, anion gap, and urea concentrations in blood of goats. However, HS had no effect on glucose, pH, Cl, hematocrit or hemoglobin values.

The deceased pCO_2 and HCO_3^- under HS conditions agree with results reported in dairy cows (Schneider et al., 1988) and goats (Hamzaoui et al., 2013a). The greater respiration rate observed in HS goats contributed to greater loss of CO_2 and lowering the carbonic acid content of the blood. To maintain the blood pH constant, HCO_3^- is transferred from blood to

urine by the kidney. The decrease in blood HCO_3^- was the same as in our previous study (-5 points approximately).

The SBO supplementation decreased ($P < 0.05$) blood pH. This change in blood pH, even significant, is of low physiological importance because the blood pH is regulated by a complex system of buffers that continuously work to maintain it slightly basic in a range of 7.35 to 7.45 in most mammals according to Constable (1999). Our blood pH values were clearly within the normal range. Supplementation with SBO increased blood NEFA concentration, which agrees with previous results in dairy cows (Bu et al., 2007; Grummer and Carroll, 1991; Petit et al., 2002; Petit, 2002). This increase in NEFA concentration was not accompanied by an increment in BHBA levels, which might indicate that NEFA were rapidly taken up by the mammary gland for fat synthesis and were not transformed to ketone bodies by the liver.

Table 5.4. Blood indicators of Murciano-Granadina dairy goats under thermal neutral (TN) and heat stress (HS) conditions. In each ambient, goats were not supplemented (C) or supplemented with 4% soybean oil (S).Values are LSM and SE of the means (SEM).

	TN		HS			Effect (*P*<)		
Item[1]	C	S	C	S	SEM	Treatment	Ration	T x R[2]
Goats, n	8	8	8	8	-	-	-	-
Na, mmol/L	151.6	150.9	146.5	146.6	1.16	0.001	0.790	0.710
K, mmol/L	3.65	3.69	3.94	3.83	0.11	0.057	0.729	0.489
Cl, mmol/L	109.6	111.8	109.6	112.6	1.53	0.777	0.106	0.777
Total CO_2, mmol/L	28.63	27.13	22.88	21.25	1.00	0.001	0.129	0.951
Urea, mg/dL	21.13	21.38	17.13	16.75	0.96	0.001	0.949	0.748
Glucose, mg/dL	55.13	53.75	56.38	55.38	1.59	0.374	0.462	0.907
Hematocrit, % PCV[3]	18.13	17.63	18.13	17.25	0.82	0.821	0.410	0.821
Hemoglobin, g/dL	6.16	6.00	6.16	5.88	0.28	0.826	0.431	0.826
pH	7.46	7.45	7.47	7.44	0.01	0.511	0.038	0.205
pCO_2[4], mmHg	38.95	37.83	29.65	29.68	1.58	0.001	0.711	0.692
HCO_3^-, mmol/L	27.33	26.03	21.96	20.29	0.99	0.001	0.146	0.852
Base excess, mmol/L	3.25	1.75	-1.88	-3.88	1.03	0.001	0.102	0.811
Anion gap, mmol/L	17.00	16.88	18.75	17.75	0.61	0.039	0.36	0.476
NEFA, mmol/L	0.066	0.110	0.086	0.119	0.023	0.202	0.025	0.731
BHBA, mmol/L	0.652	0.609	0.959	0.716	0.071	0.168	0.224	0.493

[1] Obtained values in the morning before changing from night (30°C and 40% humidity; THI = 77) to day (37°C and 40% humidity; THI = 85) conditions.[2] Treatment (T) × Ration (R) interaction.[3] Packed cell volume.[4] Partial pressure of CO_2.

5.5 CONCLUSIONS

Heat stress caused losses in milk yield and milk components in dairy goats. The supplementation with 4% soybean oil increased milk fat, trans-vaccenic acid and conjugated linoleic acid (*cis*-9, *trans*-11 isomer) without affecting milk protein. Both heat stress and soybean oil increased the percentage of long chain fatty acids in milk and decreased the atherogenicity index. There was no interaction between oil supplementation and heat stress for most of the studied variables, indicating the dairy goats responded to soybean oil in a similar manner independent of the ambient temperature.

CHAPTER 6

Effects of supplementation with propylene glycol in heat-stressed dairy goats

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6.1 ABSTRACT

We hypothesized that supplementation with propylene glycol (PG) would increase blood glucose and spare amino acids for milk protein synthesis rather than glucose production. To test this hypothesis, we used 8 multiparous Murciano-Granadina dairy goats (40.8 ± 1.1 kg BW; 84 ± 1 DIM) individually kept in metabolic cages. The design was a replicated 4 × 4 Latin square of 4 periods; 21 d each (14 d adaptation, 5 d for measurements, and 2 d of transition). Goats were allocated to one of 4 treatments in a 2 × 2 factorial arrangement. Factors were no propylene glycol (C) or 5% of propylene glycol (PG), and thermal neutral (TN; 15 to 20°C) or heat stress (HS; 12 h/d at 37°C and 12 h/d at 30°C) conditions. This resulted in 4 treatment combinations: TN-C, TN-PG, HS-C, and HS-PG. Feed intake, rectal temperature, respiration rate, milk yield, milk composition, and blood parameters were measured. Compared to TN, HS goats had lower ($P < 0.05$ to 0.10) feed intake, fat corrected milk, milk fat, milk protein, and milk lactose. The supplementation with 5% of PG increased blood glucose ($P < 0.05$) and tended to increase ($P< 0.10$) blood insulin, but dry matter intake tended to decrease ($P < 0.10$). Furthermore, blood NEFA and β-hydroxybutyrate acid (BHBA) decreased ($P < 0.05$) by PG. In conclusion, supplementation of heat-stressed dairy goats with propylene glycol did not affect milk yield or milk protein content, and caused milk fat depression syndrome.

6.2 INTRODUCTION

Compared to thermal-neutral (TN) conditions, dairy goats under heat stress (HS) conditions had lower DMI and milk yield with systematic depressed milk protein content (Hamzaoui et al., 2013a, 2013b, 2014). This syndrome of decreased milk protein content under HS conditions was widely observed also in dairy cows (Rhoads et al., 2009; Baumgard and Rhoads, 2013). This reduction in milk yield and protein is accompanied by a down-regulation in milk protein genes, and up-regulation of apoptosis genes in the mammary gland (Collier et al., 2006; Salama et al., 2014).

Under HS conditions, DMI decreased by 25 to 40%, but milk yield decreased by only 3 to 10% (Hamzaoui et al., 2013a, b). Unexpectedly, this dramatic reduction in DMI was not accompanied by body fat mobilization, as blood NEFA levels did not vary between HS and TN goats (Baumgard and Rhoads, 2013; Hamzaoui et al., 2013a). The notable BW losses and the no change in blood glucose levels under HS might indicate that HS-goats catabolize their muscles (protein mobilization) to keep glucose levels and milk production. Consequently, it is logical to think that if HS-goats are fed with gluconegenic component, they would reduce the muscle degradation and spare AA for milk protein synthesis.

Propylene glycol (PG) is a glucogenic precursor that is either rapidly absorbed from the rumen and converted to glucose, or partially metabolized to propionate in the rumen before being absorbed (Nielsen and Ingvartsen, 2004). Moreover, it provides substrates for gluconeogenesis and induces insulin resistance in peripheral tissues to spare glucose for milk synthesis (Kristensen and Raun, 2007). Several studies have shown that an oral drench of PG is effective in increasing glucose and decreasing NEFA and BHBA in early lactating dairy cows (Goff and Horst, 2001; Linke et al., 2004; Nielsen and Ingvartsen, 2004; Osman et al., 2008; Rizos et al., 2008).

The aim of this study was to test the effectiveness of propylene glycol in dairy goats under heat stress conditions. We expected that feeding propylene glycol would increase blood glucose and spare amino acids for milk protein synthesis.

6.3 MATERIALS AND METHODS

6.3.1 Animal and Management Conditions

Animal care conditions and management practices agreed with the procedures stated by the Ethical Committee of Animal and Human Experimentation of the Universitat Autònoma de Barcelona (Bellaterra, Spain; CEEAH reference 09/771) and the codes of recommendations for the welfare of livestock of the Ministry of Agriculture, Food and Environment of Spain (Madrid).

Eight multiparous Murciano-granadina dairy goats (84 ± 1 DIM, 2.00 ± 0.04 L/d of milk yield; 40.8 ± 1.1 kg BW) with healthy and symmetrical udders were used from the herd of the experimental farm of the Universitat Autonoma de Barcelona. Goats were kept in metabolic cages and were used in a replicated 4 × 4 Latin square design with 4 periods; 21 d each (14 d adaptation, 5 d for measurements and 2 d transition between periods). When goats were switched from TN to HS conditions, a transition period of 2 d was allowed (1 d at 25°C, 1 d at 30°C), but the change from HS to TN was abrupt. Goats were allocated to one of 4 treatments in a 2 × 2 factorial arrangement. Factors were no propylene glycol (C) or 5% of propylene glycol (PG), and thermal neutral (TN; 15 to 20°C) or heat stress (HS; 12 h/d at 37°C and 12 h/d at 30°C) conditions. This resulted in 4 treatment combinations: TN-C, TN-PG, HS-C, and HS-PG. The humidity was maintained at 40 ± 5%. Data of environmental temperature and humidity were recorded every 10 min throughout the experiment by a data logger (Opus 10, Lufft, Fellbach, Germany) and THI values were calculated according to NRC (1971).

The daily ration for all goats consisted of TMR (Alfalfa hay 60.4%, ground barley grain 15%, beet pulp 9.1%, ground corn grain 7.5%, soybean meal 3%, sunflower meal 3%, molasses 1%, salt 0.6%, sodium bicarbonate 0.2%, and CVM for goats 0.2%). Chemical composition and nutritive value are shown in Table 6.1. Mineral and vitamin blocks were freely available for each goat. The PG (1, 2-Propilenglicol – USP, LABIANA Life Sciences S.A.U. Terrassa, Barcelona, Spain) was thoroughly mixed daily with the ration. Equivalent amount of water was mixed with the control rations.

Goats were milked once daily (0800 h) with a portable milking machine (Westfalia-separator Ibérica, Granollers, Spain) with recorded jars (3L ± 5%). Milking was conducted at a vacuum pressure of 42 kPa, a pulsation rate of 90 pulses/min, and a pulsation ratio of 66%. The milking routine included cluster attachment without udder preparation or teat cleaning,

machine milking, machine stripping before cluster removal, and teat dipping in an iodine solution (P3-ioshield, Ecolab Hispano-Portuguesa, Barcelona, Spain).

6.3.2 Sample Collection, Analyses, and Measurements

6.3.2.1 Body Temperature and Respiration Rate

Rectal temperatures and respiration rates were recorded at 0800, 1200, and 1700 h. The rectal temperature was measured by a digital clinical thermometer (Model ICO Technology "mini color" Barcelona, Spain; 32 to 43.9 ± 0.1ºC). The respiration rate was measured by counting the inhalations and exhalations for 60 s with the aid of a chronometer.

6.3.2.2 Feed Intake and Water Consumption

Feed intake and water consumption (accuracy: ± 20 g) were recorded daily throughout the experiment. Trays with saw dust were put below the drinking troughs and weighted twice daily to take into account water wastes. Feed samples were collected daily during measurement days of each period and were ground through a 1-mm stainless steel screen, and then analyzed for DM, ADF, NDF, and ash content according to analytical standard methods (AOAC International, 2003). The Dumas method (Leco analyzer , LECO Corp., St. Joseph, MI) was used for N determinations and CP was calculated as percentage of N × 6.25.

6.3.2.3 Milk Yield and Milk Composition

Milk yield of individual goats was recorded daily throughout the experiment. A milk sample of approximately 50 mL was collected for two consecutive days during the measurement days of each period and preserved with an antimicrobial tablet (Bronopol, Broad Spectrum Microtabs II, D&F Control Systems Inc., San Ramon, CA) at 4°C until analysis. Milk samples were analyzed with a near-infrared spectrometer (Foss NIRSystems 5000, Foss, Hillerød, Denmark) for contents of fat, protein (N × 6.38), and lactose.

Table 6.1. Chemical composition and nutritive value (DM basis) of the total mixed ration used for dairy goats.

Item	Total mixed ration
Component, %	
Dry matter	89.50
Organic matter	87.59
Crude protein	16.42
Neutral detergent fiber	35.78
Acid detergent fiber	24.61
Nutritive value[1]	
UEm,[2] /kg	0.71
UFL,[3] /kg	0.82
NE_L, Mcal/kg	1.40
PDIE,[4] g/kg	100
PDIN,[5] g/kg	116
PDIA,[6] g/kg	50
Ca, g/kg	10.55
P, g/kg	2.86

[1] Calculated according to Institut National de la Recherche Agronomique (INRA, 2007). [2] Fill units for sheep (1 UEm = 1 kg of reference grass DM). [3]Feed units for lactation (1 UFL = 1.7 Mcal of NEL). [4] Protein digested in the small intestine supplied by microbial protein from rumen-fermented OM. [5] Protein digested in the small intestine supplied by microbial protein from RDP. [6] Protein digested in the small intestine supplied by RUP.

6.3.2.4 Blood Measures

Blood samples were taken at d 5 of each measurement period from the jugular vein into vacutainers (Venoject, Leuven, Belgium) before the morning feeding. Plasma was obtained by centrifugation of whole blood for 15 min at 1500 × *g*, and stored at -20°C for the NEFA, β-hydroxybutyrate acid (BHBA), insulin, and lactate analyses. The NEFA were analyzed by the colorimetric enzymatic test ACS-ACOD method using a commercial kit (Wako Chemicals, Neuss, Germany). The BHBA was determined by kinetic enzymatic method using commercial kit (RANBUT, Randox®, UK). Insulin was measured by ELISA type sandwich using the commercial kit (Mercodia Ovine Insulin ELISA, Mercodia®; Switzerland) and the lactate was determined by enzymatic method (Olympus System Reagent®, Beckman Coulter®, Ireland). Moreover, whole blood was used for the glucose, urea, and creatinine determination. A single drop of whole blood was applied to disposable cartridges containing

biochemical and silicon chip technology (iSTAT CG8+ Crea cartridges, Abbott Point of Care Inc., Princeton, NJ). Then, the cartridge was inserted into an i-STAT handheld analyzer, and the results were obtained.

6.3.3 Statistical Analyses

Data were analyzed by the PROC MIXED for repeated measurements of SAS version 9.1.3 (SAS Institute Inc., Cary, NC). The statistical mixed model contained the fixed effects of the treatment (TN-C, TN-PG, HS-C and HS-PG) and period; the random effect of the animal; the interactions treatment × period; and the residual error. The model took into account the possible carryover effects of previous HS periods through the treatment × period interaction. Data of performances (i.e., intake, water, milk yield and milk composition) and physiological indicators (i.e., rectal temperature and respiratory rate) were analyzed on a daily basis.

6.4 RESULTS AND DISCUSSION

6.4.1 Rectal Temperature and Respiration Rate

As expected, HS goats showed a greater ($P < 0.001$) rectal temperatures and respiration rates than TN goats (Table 6.2). According to the review of Nielsen and Ingvartsen (2004), farmers and veterinarians in Denmark have experienced that some cows had rapid shallow breathing, ataxia, salivation, somnolence and depression when adding PG to the feed ration. Rapid shallow breathing, ataxia, and salivation have also been described in horses, which were accidentally given 3–4 kg PG (Dorman and Haschek, 1991; McClanahan et al., 1998). The dose used in the current study (5%) was not toxic and no effect of PG supplementation on rectal temperature or respiration rate was detected (Table 6.2).

6.4.2 Feed Intake and Water Consumption

The DMI and water consumption data of TN and HS dairy goats with or without 5% of PG supplementation mixed with TMR are shown in Table 6.2. The decrease ($P < 0.01$) in DMI by the effect of HS was 34% on average, and HS goats almost doubled (+185%) their water consumption. Moreover, PG tended ($P < 0.10$) to decrease DMI in both TN and HS goats with no effect on water consumption.

Table 6.2. Intake, milk production, FCM, milk composition, rectal temperature and respiration rate of Murciano-Granadina dairy goats under thermal neutral (TN) and heat stress (HS) conditions. In each ambient, goats were not supplemented (C) or supplemented with 5% of propylene glycol (PG). Values are LSM and SE of the means (SEM).

	TN		HS			Effect (*P* - value)		
Item	C	PG	C	PG	SEM	Temperature	Ration	T x R[1]
Goats, n	8	8	8	8	-	-	-	-
Intake, kg DM/d	2.34	2.19	1.59	1.38	0.07	0.001	0.060	0.776
Water intake, L/d	5.99	5.84	11.16	10.74	1.07	0.001	0.797	0.900
BW change, kg	2.9	3.2	-4.1	-3.5	0.51	0.001	0.117	0.723
Milk, L/d	1.86	1.80	1.79	1.66	0.18	0.210	0.258	0.614
3.5 % FCM[2], L/d	2.12	1.78	1.85	1.48	0.16	0.002	0.001	0.856
Milk composition, %								
Fat	4.43	3.46	3.78	2.89	0.15	0.009	0.002	0.856
Protein	3.55	3.54	3.14	3.15	0.15	0.074	0.994	0.963
Lactose	4.47	4.46	4.31	4.29	0.06	0.064	0.886	0.980
Rectal temperature, ºC								
0800	38.7	38.8	39.3	39.2	0.07	0.001	0.775	0.223
1200	39.0	38.9	39.8	39.8	0.09	0.001	0.580	0.937
1700	39.1	39.1	40.2	40	0.10	0.001	0.609	0.511
Respiration rate, breaths/min								
0800	26.6	27	81.1	80.7	2.64	0.001	0.996	0.885
1200	31.7	31.5	116.5	108.4	3.97	0.001	0.315	0.342
1700	34.4	33.3	136	131.7	3.01	0.001	0.388	0.607

[1]Interaction of Temperature (T) × ration (R). [2] Fat corrected milk at 3.5%; FCM = L × [0.432 + 0.162 × (fat %)], being L liters of milk yield.

The tendency of decreasing DMI (-10% on average) by the effect of the PG supplementation had been reported by Chibisa et al. (2008) in dairy cows when PG was mixed with the TMR (-0.4 to -2.1 kg/d). Furthermore, Miyoshi et al. (2001) observed that lactating cows decreased their feed intake after 1 to 2 d of top-dressing 518 g/d of PG. Similarly, Dhiman et al. (1993) reported a significant reduction in DMI in mid-lactating cows fed 688 g/d of PG added to TMR. The reduction in DMI could be explained by the unpalatability of the PG (Johnson, 1954; Girschewski et al., 1977; Nielsen and Ingvartsen, 2004). Miyoshi et al. (2001) suggested that the most effective way of allocating PG without

affecting DMI is by drenching or by mixing PG into the concentrates, which may include molasses or other attractive-flavor additives. The PG is an additive with a high NE_L content (4 Mcal/kg) as reported by Miyoshi et al. (2001), which abolishes the effect of reduced DMI. In the current study, DMI (and NE) intakes decreased by 0.15 kg (-0.14 Mcal) and 0.21 kg (-0.20 Mcal) in TN-PG and HS-PG goats, respectively. On the other hand, TN-PG and HS-PG goats received 122 g (+0.49 Mcal) and 77 g (+0.31 Mcal) of PG on average, respectively. It means that although PG goats had lower DMI, they in fact had greater NE intake.

6.4.3 Milk yield and milk composition

Milk yield, FCM and milk composition (fat, protein and lactose content) data of TN and HS dairy goats with or without PG supplementation are shown in Table 6.2. The HS numerically ($P = 0.210$) decreased milk yield by 6%, which is similar to what observed in dairy goats by Hamzaoui et al. (2013b). Moreover, this loss in milk yield is in the range (3 to 13%) reported by Salama et al. (2014) for HS-dairy goats. However, milk fat (and consequently FCM), milk protein, and milk lactose decreased ($P < 0.05$) or tended ($P < 0.10$) to decrease by HS. Losses in milk components due to HS have been observed in dairy cows (Rhoads et al., 2009; Shwartz et al., 2009; Wheelock et al., 2010) and ewes (Finocchiaro et al., 2005).

The PG supplementation did not affect milk yield, but decreased ($P < 0.01$) milk fat content, resulting in lower ($P < 0.01$) FCM. No effect of PG on milk protein or lactose was detected. Our hypothesis was that supplementation with PG would increase blood glucose and spare AA for milk protein synthesis. The PG had a positive effect on blood glucose (see later), increased energy intake (see above), decreased milk fat (sparing more energy), but neither milk yield nor milk protein were improved by PG. This saved energy was clearly deposited in body and was not used for milk production as variation in BW tended ($P = 0.117$) to be positive in TN and HS goats when PG was supplemented (Table 6.2).

The milk fat depression (-23%) suffered by PG-goats agrees with the results of Fisher et al. (1973) in dairy cows, although this negative effect was not detected by others (Miyoshi et al., 2001; Moallem et al., 2007; Chibisa et al., 2008). The reduced milk fat by PG could be due to, first, the decrease in plasma NEFA (see later) since lowered NEFA concentrations lead to decreased NEFA-uptake by the mammary gland (Emery and Herdt, 1991; Nielsen and Riis, 1993); and second, PG could lower proportion of acetate in the rumen (Grummer et al., 1994;

Shingfield et al., 2002) which may reduce the amount of acetate available for de novo FA synthesis in the mammary gland.

6.4.3 Blood measures

Blood insulin, glucose, urea, creatinine, NEFA, BHBA, and lactate of TN and HS goats supplemented or not with 5% of PG are shown in Table 6.3. Except blood urea, HS did not affect the studied blood measures. The decrease in blood urea concentration could be explained by less DMI resulting in less nitrogen absorption by the intestine. Despite the reduced feed intake, HS goats kept similar glucose level to TN goats and did not mobilize body fat reserves (no change in NEFA and BHBA values). In dairy cows, Rhoads et al. (2009) and Baumgard and Rhoads (2013) reported that blood NEFA did not vary due to HS (similar to goats), but blood insulin levels were significantly increased (such an increase was not detected in goats). Baumgard and Rhoads (2013) indicated in their review on the effects of HS on metabolism and energetics that blood lactate levels are consistently elevated in many HS models, including cattle. The origin of this lactate is unknown but may include the gastrointestinal tract and muscle. In our goats, no effect of HS on blood lactate was observed.

Table 6.3. Blood metabolites in Murciano-Granadina dairy goats under thermal neutral (TN) and heat stress (HS) conditions. In each ambient, goats were not supplemented (C) or supplemented with 5% of propylene glycol (PG). Values are LSM and SE of the means (SEM).

	TN		HS			Effect (*P*- value)		
Item	C	PG	C	PG	SEM	Temperature	Ration	T x R[1]
Goats, n	8	8	8	8	-	-	-	-
Insulin, µg/L	1.14	1.54	1.03	1.43	0.16	0.636	0.091	0.998
Glucose, mg/dL	56.14	61.71	56.14	57.57	1.55	0.120	0.012	0.120
BUN, mg/dL	25.71	23.86	18.43	18.14	2.81	0.007	0.628	0.722
Creatinine, mg/dL	0.49	0.52	0.48	0.58	0.04	0.484	0.044	0.274
NEFA, mmol/L	0.10	0.06	0.07	0.03	0.01	0.116	0.021	0.818
BHBA, mmol/L	0.65	0.48	0.77	0.48	0.05	0.368	0.002	0.397
Lactate, mmol/L	0.51	0.52	0.46	0.51	0.03	0.490	0.446	0.602

[1] Interaction of temperature (T) × ration (R).

The PG supplementation tended ($P < 0.10$) to increase blood insulin as a response of the increment ($P < 0.05$) in blood glucose levels (Table 6.3). The PG is a glucogenic precursor that is either rapidly absorbed from the rumen and converted to glucose, or partially metabolized to propionate in the rumen before being absorbed (Nielsen and Ingvartsen, 2004). As pointed out by Kristensen and Raun (2007), a portion of PG is escaped from the rumen of cows and uptaken by liver to produce lactate. Lactate, and not glucose, appeared to be the main product of hepatic metabolism of PG. Nevertheless, in the current study no increase in blood lactate was observed when PG was fed to goats, probably indicating that PG was totally fermented in the rumen.

Despite the positive effect of glucose on milk yield (Rhoads et al., 2009) and insulin on milk protein synthesis (Menzies et al., 2009), no changes in milk yield or milk protein were observed in the current study (see above). Glucose increased by PG in TN goats but not in HS goats (P of interaction = 0.120). We supposed that HS goats are in need of glucose for milk production. However, with the increment in insulin levels (+39%), it seems the glucose rapidly disappeared from blood and directed to alleviate BW losses (HS-PG goats tended to loss less BW than HS-C goats). Moreover, supplementation with PG decreased ($P < 0.05$) NEFA (-49%) and BHBA (-32%) in plasma. Insulin has a lipogenic effect (Rodbell, 1964; Vinten et al., 1976) and directs energy towards body tissues rather than the mammary gland, resulting in lower levels of circulating NEFA and BHBA. Finally, creatinine concentration was greater ($P< 0.05$) in goats fed PG. It is not clear why creatinine increased by PG in our goats, but human patients treated with Lorazepam infusion (containing PG as a solvent) had greater blood creatinine (Yaucher et al., 2003). Those authors proposed that this effect could result from proximal renal tubular cell injury by PG.

6.5 CONCLUSIONS

Milk protein depression is a constant constrain observed in milk produced from heat-stressed animals. We hypothesized that propylene glycol supplementation to heat-stressed goats would increase blood glucose levels and spares amino acids for milk protein synthesis. In contrary to our hypothesis, propylene glycol did not affect milk yield or milk protein content, despite the increment in circulating blood glucose and insulin. Moreover, a strong milk fat depression was detected, which could be related to the decrease in NEFA and BHBA levels in blood.

CHAPTER 7

Conclusions

CHAPTER 7

CONCLUSIOS

In accordance with our objectives, the current thesis allowed us in the first section to measure responses of dairy goats to heat stress (Chapters 3 and 4) and in the second section to evaluate soybean oil and propylene glycol as supplements to improve milk quality (Chapters 5 and 6).

Section 1: Responses of dairy goats to heat stress:

- The impact of heat stress on milk production varies according to the sage of lactation, being greater at earlier than at later stages.
- Significant decreases in feed intake were observed (-25 to -40%), but milk yield losses were only -3 to -10%.
- Heat stress had a strong negative effect on milk protein, and to a lower extent, on milk fat.
- Digestibility coefficients were relatively improved by heat stress, which might partially compensate for the reduced feed intake.
- Despite the decrease in feed intake, heat-stressed goats were able to maintain normal blood glucose values without fat reserves mobilization.
- Lipid tissue of dairy goats became insensitive to lipolytic hormones, but had the same response to the lipogenic signals, which explains the lack of fat mobilization under heat stress.
- The pancreas of heat-stressed goats secreted lower insulin amounts in response to glucose administration. This finding might be a mechanism by which goats keep blood glucose levels in high ambient temperatures, even with the clear reduction in feed intake.
- Blood pH was kept within the normal range by more clearance of bicarbonate from blood.
- Heat stress had no effect on eating bouts, but the reduced feed intake observed during heat stress was due to the shorter time of each eating bout.

- The increment in water consumption in the hot ambient was due the elevated number of drinking bouts rather than the duration of drinking bouts.

Section 2: Supplementation the ration of heat-stressed goats with soybean oil and propylene glycol:

- The supplementation with 4% soybean oil increased milk fat, trans-vaccenic acid and conjugated linoleic acid (cis-9, trans-11 isomer).
- The magnitude of response to soybean oil was similar in control as well heat stress conditions.
- No negative effect was observed on milk protein when soybean oil was supplemented.
- Both heat stress and soybean oil increased the percentage of long chain fatty acids in milk and decreased the atherogenicity index.
- The supplementation with propylene glycol had similar effects in thermo-neutral and heat stress conditions.
- Blood glucose and insulin levels increased by propylene glycol, but no changes were observed in milk protein.
- It seems that this increment in glucose was directed to body stores rather than its use by the mammary gland.
- Goats with propylene glycol had lower feed intake with a strong milk fat depression, which could be related to the decrease in non-esterified fatty acids and β-hydroxybutyrate levels in blood.

CHAPTER 8

References

CHAPTER 8

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