Effects of thermal stress on dairy goats and their offspring

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Efectos del estrés térmico en cabras lecheras y su descendencia

Efectes de l'estrès tèrmic en cabres lleteres i la seva descendència

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- **W. Coloma-García**, N. Mehaba, A. A. K. Salama, X. Such, and G. Caja (2018). Effects of prenatal heat stress on the emotional reactivity and behavioral reactions of female dairy goat kids. EAAP Book of Abstracts, vol. 24, p. 115, Dubrovnik (Croatia).
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- **W. Coloma-García**, N. Mehaba, A. A. K. Salama, X. Such, and G. Caja (2018). Effects of prenatal heat stress on the emotional reactivity and behavioral reactions of female dairy goat kids. Journal of Dairy Science, vol. 101, suppl. 2, p. 213.
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LIST OF ABBREVIATIONS

ADF	Acid detergent fiber
ADG	Average daily gain
ANOVA	Analysis of variance
AT	Arena test
BCS	Body condition score
BHBA	Beta-hydroxybutyrate acid
BUN	Blood urine nitrogen
BW	Body weight
CEAAH	Ethical committee on human and animal experimentation
CP	Crude protein
C$_P$G	Cytosine-phosphate-Guanine island
CS	Cold stress
DIM	Days in milking
DM	Dry matter
DMI	Dry matter intake
EC	Epinephrine challenge
FA	Fatty acid
FCR	Feed conversion ratio
FFA	Free fatty acid
GLM	Generalized linear model
GLMM	Generalized linear mixed model
GH	Growth hormone
GHR	Growth hormone receptor
GR	Glucocorticoid receptor
GTT	Glucose tolerance test
HPA	Hypothalamus-pituitary-adrenal axis
HS	Heat stress
HSP	Heat-shock protein
IC	Insulin challenge
IGF-1	Insulin-like growth factor
IUGR	Intrauterine-growth retarded
IUHS	*In utero* thermo-neutral
IUTN	*In utero* heat stress
LCT	Lower critical temperature
LT	Low temperature
MCT	Maximum critical temperature
MR	Mineralcorticoid receptor
NEFA	Non-esterified fatty acid
NDF	Neutral detergent fiber
NOT	Novel object test
PI	Placental insufficiency
RH	Relative humidity
RR	Respiration rate
RT	Rectal temperature
SCC	Somatic cell counts
SED	Standard error of the difference
THI	Temperature-humidity index
Tlc	Lower critical temperature
TMR	Total mixed ration
TN	Thermal neutrality

Trt	Treatment	WI	Water intake
Tuc	Upper critical temperature	VFA	Volatile fatty acid

Summary

In the present thesis, 3 experiments were carried out using Murciano-Granadina dairy goats under low temperature (LT; Exp. 1) and heat stress (HS; Exp. 2 and 3) conditions in order to evaluate the physiological, metabolic, productive and behavioral responses. In Exp. 1, 8 dairy goats in mid-lactation were randomly allocated into 2 groups: thermal-neutral (TN; 15 to 20°C) and LT (-3 to 6°C) in a crossover design with 2 treatments in 2 periods (21 d each). Body weight, feed intake, water consumption, milk production, rectal temperature (RT), respiratory rate (RR), as well as blood insulin, glucose, non-esterified fatty acids (NEFA), beta-hydroxybutyrate (BHB), cholesterol and triglycerides were measured. Compared to TN goats, LT goats had similar feed intake, but lower ($P < 0.05$) water consumption (−23%), RR (−6 breaths/min) and RT (−0.32°C). Milk of LT goats had greater ($P < 0.05$) contents of protein (+10%), fat (+12%) and lactose (+4%) than TN goats. The LT goats lost −0.45kg BW, whereas TN goats gained 2.2 kg ($P < 0.05$). Values of blood glucose, NEFA, hematocrit and hemoglobin increased ($P < 0.05$) by LT, whereas BHB and triglycerides decreased ($P < 0.05$). In Exp. 2, 30 dairy goats were exposed to 2 treatments: TN (15 to 20°C), and HS (30 to 37°C) from 12 days before mating to day 45 of gestation. Feed intake, RT and RR were recorded. After kidding, litter size, birth weight and weaning weight of kids were registered. At 27 ± 4 days old, female kids exposed to in utero TN (IUTN; n = 16) and HS (IUHS; n = 10) were subjected to 2 tests: arena test (AT) and novel object test (NOT). In both tests, kids were entered a 4x4 m^2 arena and the distance travel, number of squares entered, jumps and sniffs of the arena were recorded. At 8 months old, a subset of growing goats (n = 8) from each group (IUTN and IUHS) were exposed to 2 environmental conditions in 2 consecutive periods: a TN period for 7 d at 15-20 °C (P1), and then HS for 21 d at 30-37°C (P2). Feeding behavior and other activities (drinking, exploring, and grooming) and resting behaviors, as well as posture were recorded. Compared with TN, gestation length was shortened by HS (-3d; $P < 0.01$). In the AT, IUHS kids showed a lower number of sniffs ($P < 0.01$) than IUTN. Further, during HS (P2), both IUTN and IUHS goats spent more time resting, panting and drinking ($P < 0.001$) with no differences between both groups. In Exp. 3, the same goats (IUTN; n =8, and IUHS; n = 8) used for HS behavior in Exp. 2 were used. Body weight, feed intake, water consumption, RT, RR as well as blood glucose, insulin, NEFA, BHB, cholesterol and triglycerides were measured. In addition, 4 goats from each treatment were subjected to a glucose tolerance test (GTT), insulin tolerance test (ITT), and an epinephrine challenge, each one performed once during P1 and P2. Regardless the in utero thermal treatment, HS resulted in increased ($P < 0.01$) RT, RR and water consumption, but decreased ($P < 0.05$) body weight gain. However, IUHS goats needed 10% less ($P < 0.05$) RR to keep similar RT to IUTN goats. Compared to IUTN, IUHS goats had greater glucose area under curve at 45 min ($P = 0.077$) and 90 min ($P = 0.056$), and lower ($P < 0.05$) insulin clearance rate in response to the GTT. In conclusion, LT goats had similar DM intake to TN goats, but their milk contained greater fat and protein. The LT goats covered the extra energy needed for heat production by mobilizing body fat reserves as indicted by greater blood NEFA levels. Heat stress during the first third of pregnancy shortened pregnancy length,

and modified some behavioral, physiological and metabolic responses to various stimulators, including HS during the postnatal life.

Resumen

En la presente tesis, se realizaron 3 experimentos con cabras lecheras Murciano-Granadinas en condiciones de baja temperatura (LT; Exp. 1) y estrés por calor (HS; Exp. 2 y 3), para evaluar las condiciones fisiológicas, metabólicas, productivas y de respuesta conductual. En la exp. 1, 8 cabras lecheras en mitad de lactación se asignaron aleatoriamente en 2 grupos: termoneutro (TN; 15 a 20 °C) y LT (-3 a 6 °C), en un diseño cruzado de 2 tratamientos en 2 períodos (21 d cada uno). Se midió el peso corporal, ingesta de alimento, consumo de agua, producción de leche, temperatura rectal (RT), frecuencia respiratoria (RR), así como insulina en sangre, glucosa, ácidos grasos no esterificados (NEFA), beta-hidroxibutirato (BHB), colesterol y los triglicéridos. En comparación con las cabras TN, las cabras LT presentaron un consumo de alimento similar, pero un menor (−23%) consumo de agua (*P* <0.05), RR (−6 respiraciones / min) y RT (−0.32 °C). La leche de las cabras LT tuvo un mayor contenido (*P* <0.05) en proteína (+ 10%), grasa (+ 12%) y lactosa (+ 4%) que las cabras TN. Las cabras LT perdieron −0.45 kg BW, mientras que las cabras TN ganaron 2.2 kg (*P* <0.05). Los valores de glucosa en sangre, NEFA, hematocrito y hemoglobina aumentaron (*P* <0.05) en las LT, mientras que el BHB y los triglicéridos disminuyeron (*P* <0.05). En exp. 2, 30 cabras lecheras fueron expuestas a 2 tratamientos distintos: TN (15 a 20 °C) y HS (30 a 37 °C) desde los 12 días antes de la cubrición hasta el día 45 de gestación. Se registró la ingesta de alimento, RT y RR. Después del parto, se controló el tamaño de la camada, el peso al nacimiento y el peso al destete de las crías. A los 27 ± 4 días, las cabritas expuestas a TN en el útero (IUTN; n = 16) y HS (IUHS; n = 10) se sometieron a 2 pruebas: una prueba en espacio cerrado (Arena Test, AT) y una prueba de presencia de objeto nuevo (NO). En ambas pruebas, se introdujo a las crías en un espacio de 4x4 m^2 y se registró la distancia recorrida, el número de cuadrados pisados, los saltos y el olfateo del espacio. A los 8 meses de edad, un subconjunto de cabras en crecimiento (n = 8) de cada grupo (IUTN e IUHS) fueron expuestas a 2 condiciones ambientales en 2 períodos consecutivos: un período TN durante 7 días a 15-20 °C (P1), y luego HS durante 21 días a 30-37 °C (P2). Se registró el comportamiento alimentario y otras actividades (beber, explorar y arreglarse), y el comportamiento de descanso, así como la posición. En comparación con TN, la duración de la gestación se acortó en HS (-3d; *P* <0.01). En AT, las crías de IUHS mostraron una menor actividad de olfateo (*P* <0.01) que las IUTN. Además, durante HS (P2), las cabras IUTN y IUHS pasaron más tiempo descansando, jadeando y bebiendo (*P* <0.001), sin diferencias entre ambos grupos. En exp. 3, se utilizaron las mismas cabras (IUTN; n = 8 e IUHS; n = 8) que para el comportamiento de la exp. 2. Se midió el peso corporal, la ingesta de alimento, el consumo de agua, RT, RR, así como la glucosa en sangre, la insulina, NEFA, BHB, colesterol y triglicéridos. Además, 4 cabras de cada tratamiento fueron sometidas a una prueba de tolerancia a la glucosa (GTT), prueba de tolerancia a la insulina (ITT) y una prueba de epinefrina, realizadas una vez en los períodos P1 y P2. Independientemente del tratamiento térmico en el útero, en HS aumentó (*P* <0.01) RT, RR y consumo de agua, pero disminuyó (*P* <0.05) el incremento de peso corporal. Sin embargo, las cabras IUHS necesitaron un 10% menos de RR (*P* <0.05) para mantener una RT similar a

las cabras IUTN. En comparación con IUTN, las cabras IUHS tenían una mayor área de glucosa bajo la curva de respuesta a los 45 min (*P* = 0.077) y 90 min (*P* = 0.056), y una tasa de pérdida de insulina más baja (*P* <0.05) en respuesta al GTT. En conclusión, las cabras LT presentaron una ingestión de DM similar a las cabras TN, pero su leche contenía mayor cantidad de grasa y proteína. Las cabras LT obtuvieron la energía adicional necesaria para la producción de calor mediante la movilización de las reservas de grasa corporal, como indican los mayores niveles de NEFA en la sangre. El estrés por calor durante el primer tercio de la gestación acortó la duración de la misma y modificó algunas respuestas conductuales, fisiológicas y metabólicas a distintos estímulos, incluido el HS, durante la vida postnatal.

Resum

En la present tesi, es van realitzar 3 experiments amb cabres lleteres Murciano-Granadines en condicions de baixa temperatura (LT; Exp. 1) i estrès per calor (HS; Exp. 2 i 3), per avaluar les condicions fisiològiques, metabòliques, productives i de resposta conductual. A la exp. 1, 8 cabres lleteres a meitat de lactació es van assignar aleatòriament en 2 grups: termoneutre (TN; 15 a 20 °C) i LT (-3 a 6 °C), en un disseny creuat amb 2 tractaments en 2 períodes (21 d cadascun). Es va mesurar pes corporal, ingesta d'aliment, consum d'aigua, producció de llet, temperatura rectal (RT), freqüència respiratòria (RR), així com insulina en sang, glucosa, àcids grassos no esterificats (NEFA), beta-hidroxibutirat (BHB), colesterol i els triglicèrids. En comparació amb les cabres TN, les cabres LT van presentar un consum d'aliment similar, però un menor (-23%) consum d'aigua (P <0.05), RR (-6 respiracions / min) i RT (-0.32 °C) . La llet de les cabres LT va tenir un major contingut (P <0.05) en proteïna (+ 10%), greix (+ 12%) i lactosa (+ 4%) que la de les cabres TN. Les cabres LT van perdre -0.45 kg BW, mentre que les cabres TN van guanyar 2.2 kg (P <0.05). Els valors de glucosa en sang, NEFA, hematòcrit i hemoglobina van augmentar (P <0.05) en les LT, mentre que el BHB i els triglicèrids varen disminuir (P <0.05). En la exp. 2, 30 cabres lleteres van ser exposades a 2 tractaments diferents: TN (15 a 20 °C) i HS (30 a 37 °C) des dels 12 dies abans de la cobrició fins al dia 45 de gestació. Es va mesurar la ingesta d'aliment, RT i RR. Després del part, es va controlar la prolificitat, el pes al naixement i el pes al deslletament de les cries. Als 27 ± 4 dies, les cabretes exposades a TN a l'úter (IUTN; n = 16) i HS (IUHS; n = 10) es van sotmetre a 2 proves: una prova en espai tancat (Arena Test, AT) i una prova de presència d'objecte nou (NO). En ambdues proves, es va introduir a les cries en un espai de 4x4 m^2 i es va registrar la distància recorreguda, el nombre de quadrats trepitjats, els salts i les vegades que varen ensumar en l'espai. Als 8 mesos d'edat, un subconjunt de cabres en creixement (n = 8) de cada grup (IUTN i IUHS) van ser sotmeses a 2 condicions ambientals en 2 períodes consecutius: un període TN durant 7 dies a 15-20 °C (P1), i després HS durant 21 dies a 30-37 °C (P2). Es va registrar el comportament alimentari i altres activitats (beure, explorar i arreglar-se), i el comportaments de descans, així com la posició. En comparació amb TN, la gestació es va escurçar a HS (-3d; P <0.01). En AT, les cries d'IUHS van mostrar una menor activitat de ensumar (P <0.01) que les IUTN. A més, durant HS (P2), les cabres IUTN i IUHS van passar més temps descansant, panteixant i bevent (P <0.001), sense diferències entre els dos grups. En exp. 3, es van utilitzar les mateixes cabres (IUTN; n = 8 i IUHS; n = 8) que per al comportament de la exp. 2. Es va mesurar el pes corporal, la ingesta d'aliment, el consum d'aigua, RT, RR, així com la glucosa en sang, la insulina, NEFA, BHB, colesterol i triglicèrids. A més, 4 cabres de cada tractament van ser sotmeses a una prova de tolerància a la glucosa (GTT), prova de tolerància a la insulina (ITT) i una prova d'epinefrina, realitzades una vegada en els períodes P1 i P2. Independentment del tractament tèrmic a l'úter, a HS va augmentar (P <0.01) RT, RR i consum d'aigua, però va disminuir (P <0.05) l'increment de pes corporal. No obstant això, les cabres IUHS van necessitar un 10% menys de RR (P <0.05) per mantenir una RT similar a les cabres IUTN. En comparació amb IUTN, les cabres IUHS tenien una major àrea de glucosa sota la corba de resposta als 45 min (*P* = 0.077) i 90 min (*P* = 0.056), i una taxa de pèrdua d'insulina més baixa (*P* <0.05) en resposta al GTT. En conclusió, les cabres LT van presentar una ingesta de DM similar a les cabres TN, però la seva llet contenia major quantitat de greix i proteïna. Les cabres LT van obtenir l'energia addicional necessària per a la producció de calor mitjançant la mobilització de les reserves de greix corporal, com indiquen els majors nivells de NEFA en la sang. L'estrès per calor durant el primer terç de la gestació va escurçar la durada de la mateixa i va modificar algunes respostes conductuals, fisiològiques i metabòliques a diferents estímuls, inclòs l'HS, durant la vida postnatal.

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CHAPTER 1

General introduction

The global temperatures indicate differences of more than 55°C over the different regions on Earth. The temperature of earth surface increased by 0.7-0.9°C in the last century on average, but since 1975 it was increased to 1.5-1.8°C, and is expected to continue increasing (Blunden and Arndt, 2019). Climate change has attracted the attention of the scientific community worldwide. Searching for heat-, cold- or thermal-stress in PubMed database reveals the exponential increments in research papers focused on thermal stress from 1988 to 2018 (from 409 to 4163 papers).

In livestock, and more specifically in ruminants, most of research has been performed in those regions being affected by more extreme temperatures or with more dramatic changes (Figure 1.1).

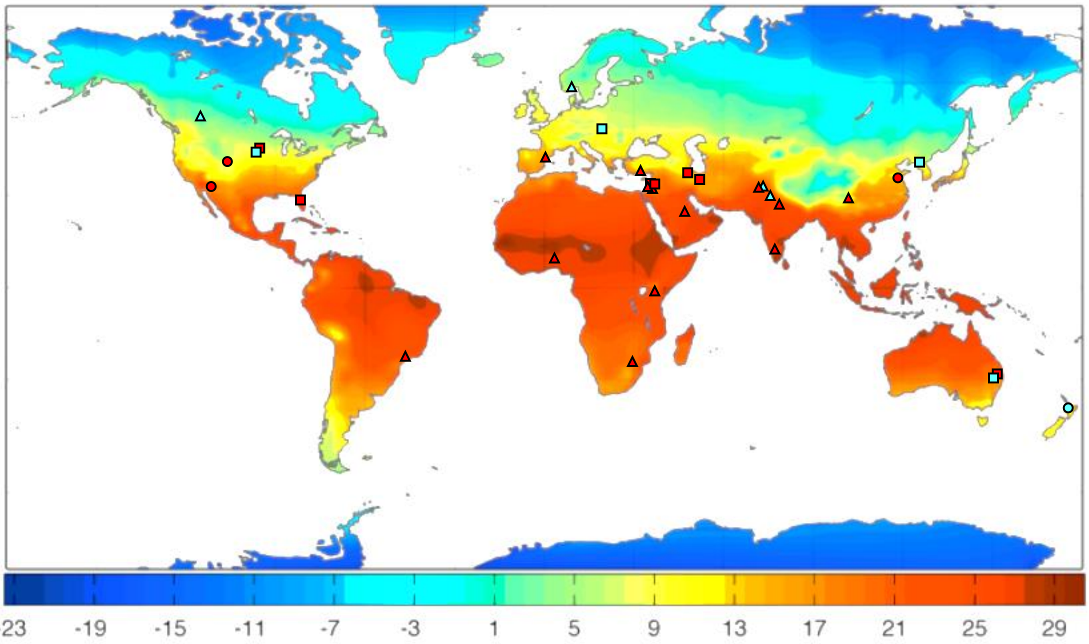

Figure 1.1. World map with average temperatures (2009; from Mourshed 2016) highlighting some of the areas where thermal stress has been assessed in ruminant livestock (reviewed in this Thesis). Legend: ● heat stress; ● cold stress; ☐ cattle; ○ sheep; and △ goat.

Extreme temperatures induce physiological changes in the animals to cope with the stress situation (Pohlin et al., 2017), and consequently cause negative impact on performance, health and welfare (Ahmed et al., 2017; Al-Dawood, 2017). Furthermore, experiencing heat stress during gestation can also affect fetal growth, which can compromise and impact the further generations in terms of reduced productive performance and suffering disorders and diseases (Akbarinejad et al., 2017; Laporta et al., 2017).

Compared to heat stress, the consequences of low ambient temperatures on livestock have received less attention, probably because large farm animals may present better thermotolerance to decreasing temperatures and remain still productive. Nevertheless, in some regions the cold temperatures and increasingly frequent cold waves have also a negative impact on the production, behavior, and welfare of animals (Kang et al., 2016; Ramón et al., 2016; Salama et al., 2016).

Compared to cows, little research has been carried out in goats to evaluate the impact of thermal stress (heat and cold) on physiology, metabolism and production. Therefore, 3 experiments were performed to expand our current knowledge on the performance of dairy goats under low ambient temperatures as well as the impact of heat stress during pregnancy on the behavior and performance of kids during the postnatal life.

CHAPTER 2

Literature review

2.1. Thermal tolerance and thermal comfort zone

Only under thermoneutral conditions it is possible to carry out an optimal function of biological processes in the animal (Brouček et al., 1991). For ruminants, it is well reported the wide range of resilience they present towards thermal distress (Battini et al., 2014), Nonetheless, when outside this range (Figure 2.1), they try to cope with the extreme temperatures by modifying their physiological, metabolic and behavioral responses in order to maintain their normal body temperature. When ambient temperature is higher than the upper critical temperature (T_{uc}), the animal dissipates heat by evaporation (sweating or panting) or by non-evaporative losses such as radiation, convection and conduction. On the contrary, when ambient temperature is lower than the lower critical temperature (T_{lc}), the animal produces heat from tissue metabolism and from fermentation, as well as resistance to heat loss by tissue (hair coat or fleece) and external insulations (from ambient air) (Freer et al., 2007).

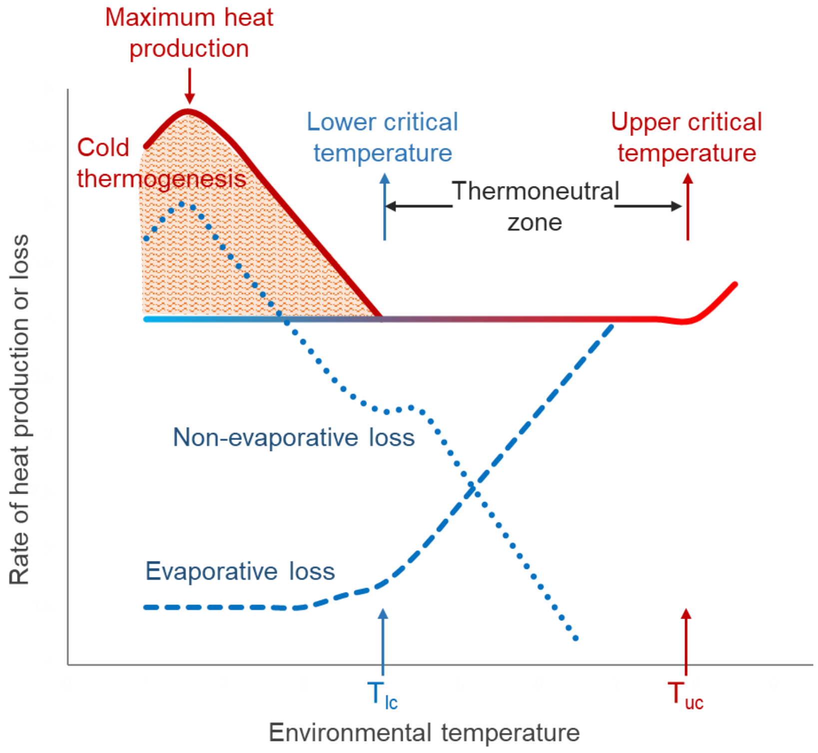

Figure 2.1. Effect of environmental temperature on thermoregulation by the animal (adapted from Freer et al., 2007).

The temperature-humidity index (THI) has been extensively used to measure the degree of heat stress (HS) in various animal species. This index was first proposed by Thom (1959), and other more recent equations to calculate the THI have been reported (Herbut et al., 2018). Despite its usefulness, the THI does not take into account wind speed and solar radiation, which are important factors under extensive production systems (Jeelani et al., 2019). Establishing thresholds of HS is not simple as the impact of HS can vary according to specie, breed, lactation stage, milk yield level, production system, geographical region, diets among other factors (Galán et al., 2018). The same could be assumed also when considering cold stress situations.

The thermoregulatory strategies and limits to thermal comfort of goats are not as well documented as dairy cows (Bøe et al., 2007; Battini et al., 2014; Yadav et al., 2016). However, goats are generally considered more rustic and adaptable, with a wide range of thermal tolerance (Sejian and Srivastava, 2010; Silanikove and Darcan, 2015; Arfuso et al., 2016), This made them spread worldwide and raised under different production systems. In this regard, the thermal comfort zone for dairy goats has been set between 55 and 70 (Salama et al., 2014; Battini et al., 2015; Pragna et al., 2018). Thus, outside this range (< 55 and > 70), goats are expected to experience changes in feed intake and production, Further, Silanikove and Darcan (2015) purposed specific THI for dairy goats at indicating higher thresholds for HS (Table 2.1). It has to be beard in mind that not always THI is calculated using the same equation.

Table 2.1. Heat stress (HS) classes based on temperature-humidity index (THI) thresholds for dairy goats (from Silanikove and Darcan, 2015).

THI	HS class
THI < 80	Normal – no effect on milk yields
80 ≤ THI < 85	Alert – modest effect on milk yields
85 ≤ THI < 90	Danger – sever effect on milk yields
THI ≥ 90	Emergency – Potential death

Goats are able to reduce the metabolism in order to reduce their maintenance and water requirements when they are under harsh environments (Silanikove, 2000). However, research indicates that goats may be affected by cold temperatures (McGregor, 2002; Bøe and Ehrlenbruch, 2013). No clear thermal comfort zone has been reported for goats, but for dairy cattle it has been set from -5 – 0ºC to 16 – 25ºC (Kadzere et al., 2002; West, 2003), and even extended to -30ºC during the peak of milk (Brouček et al., 1991).

With regard to factors that can affect THI thresholds, the age of the animals is an important one. For instance, Malabari growing goats may fail to maintain the normal body temperature at THI > 78 as indicated by Angel et al. (2018), whereas adult animals have greater resistance to HS.

Additionally, sex-specific differences may infer the response to thermal stress. Specifically, most of the works have been carried out on productive stages intrinsically involving females. Nonetheless, some authors compared males vs. females. Li et al. (2018) performed an experiment with sheep housed in a semi-open barn and exposed to extreme HS and assessed the physiological and behavioral responses. As the first signs of stress may be reported by behavior changes, they evidenced that ewes had better high-temperature tolerance than rams since the ewes exhibited greater rumination and walking.

Within species, animals show different degrees of thermal tolerance depending on the breed. These differences are even found between individuals of the same breed (Kour et al., 2017), and as expected also observed in goats (McGregor, 2002; Bøe and Ehrlenbruch, 2013; Samara et al., 2016). For instance, pure dairy breads are affected to a greater extent (Kadzere et al., 2002) while crossbreed are able to resist more extreme temperatures (El-Zarei et al., 2019). In the results observed by (Maloiy et al., 2008), heat exposure and water deprivation negatively affected to a greater extent domestic animals compared to wild and arid-originated ones.

Beyond the environmental temperatures in hot climates, air humidity and direct solar radiation also affect animal production. In this sense, the disposal of trees in open pastures has been a measure to attenuate the negative impact of HS on heifers (Lopes et al., 2016).

2.1.1. Effects of heat stress on ruminants

Heat stress has been studied with special interest in dairy animals because of its negative impact on milk yield, and the difficulty of dealing with sudden weather changes. Main responses of ruminants to heat stress are summarized in Table 2.2.

2.1.1.1. Physiological response

Rectal temperature (RT) and respiratory rate (RR) typically increase at the exposure to HS regardless of the exposure time length, production system (extensive or intensive), age, specie or breed as shown in Table 2.2.

These physiologic parameters have been considered the most reliable signs to evaluate the severity of HS in goats (Alam et al., 2011; Shilja et al., 2016). Increased RR and also RT are the first reactions after animals are exposed to environmental temperatures above the thermoneutral zone (Dangi et al., 2014; Galán et al., 2018). Measuring RR is a non-invasive and economical measurement to assess HS.

Indeed, the RR and RT can be used as a rapid tool to help detect animals better adapted to extreme temperatures. El-Zarei et al. (2019), in a study aimed to assess the potential of a crossbreeding program to improve heat tolerance, exposed pure and cross breeds (Aradi, Damascus, F1 and F2) to 45ºC for 3 d. They observed that the Damascus breed showed the highest RT, and lowest RR and expression of heat-shock proteins (HSP)[1] (70 and 90) after 3 hours of heat exposure in comparison to the Aradi breed, F1, and F2 goats. The authors of this experiment reported that the highly adaptive traits in indigenous and cross breeds related mainly with cooling through the respiratory evaporation.

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[1] Mechanism used by the animals at the cellular level to cope with high temperatures and confer transient thermal tolerance in animals exposed to thermal stress preventing the denaturation and aggregation of other proteins (Pelham, 1984).

Table 2.2. Summary of the main changes observed in domestic ruminants (sheep, goat, and cow) when exposed to heat stress conditions.

Reference	Specie	Breed	Age[1]	HS treatment[2]	CON group[3]	Main effects[4]
Li et al. (2018)[5]	Sheep	Small-tailed Han	Pubert	25 – 35ºC 5 – 35% × 2 mo	Same subject	• ↑ RT, ↑ RR, and ↑ HR • ↑ RT and ↑ RR ram > ewe • ↑ HR ewe > ram • ↑ rumination and ↑ walking ewe > ram
Maloiy et al. (2008)[5]	Sheep Goat Cattle Other bovides	Fat-tailed Turkana Zebu Adult Grant's and Thomson's gazelles, East African oryx, common wildebeest	Adult male	40.0ºC 12h/d × 18 – 21 d	Same subject	• ↓ DMI water restriction > HS • ↓ DMI domestic > wild ruminant
Sejian and Srivastava (2010)[5]	Goats	Marwari	Pubert (8-12 mo old)	40ºC 60% 4 h/d × 17 d	Same subject	• ↑ circulating glucose, cholesterol, urea and cortisol • ↓ circulating insulin
Popoola et al. (2014)	Goat	West African Dwarf	Pubert (9 mo old)	32.9ºC 47.3%	Rainy season	• ↑ RT and ↑ RR
Samara et al. (2016)	Goat	Aardi, Damascus 1:1 Aardi:Damascus 1:3 Aardi:Damascus	Pubert (6 mo old)	THI > 80 × 10 wk	Same subject	• ↑ RT and ↑ RR and ↑ skin temperature • ↑ growth crossbred > purebred
Angel et al. (2018)	Goat	Malabari	Pubert	THI 73.5 – 86.5 6 h/d × 45 d	Indoors	• ↓ BW • Degeneration in liver • ↓ mRNA GH, GHR, IGF-1, leptin, and leptin receptor in liver • Correlation between THI and IGF-1 expression
Amitha et al. (2019)	Goat	Malabari	Pubert (10-12 mo old)	THI 73.5 – 86.5 6 h/d × 45 d	Indoors	• ↓ mRNA FSHR, LHR, COX-2 • ↑ mRNA ESTR-α, PGF2- α in uterus • Degeneration in uterus
El-Zarei et al. (2019)	Goat	Aardi, Damascus F1 1:1 Aradi:Damascus F2 1:3 Aradi:Damascus	Adult (purebred) Kid (crossbred)	25 – 45ºC × 3 d	Same subject	• RT F1 and F2 (crossbred) < adult Aardi (purebred) • RR Damascus < F1 and F2 < Aardi • Htc Damascus < F1 and F2 < Aardi, • mRNA HSP in WB Aardi > F1, F2 and Damascus
Dangi et al. (2014)	Goat	Barbari	Adult (4-5 yr old)	41.0ºC»45.0ºC THI > 90 3 h/d each × 6 d	Same subject	• ↑ RT and ↑ RR • ↑ mRNA HSP (as of 1 h) • Correlation between THI, RT, RR and HSP expression

Table 2.2. (Continued)

Reference	Specie	Breed	Age[1]	HS treatment[2]	CTR group[3]	Main effects
Kaliber et al. (2016)[5]	Goat	3:1 German Fawn:Hair Goat	Adult (3 yr old)	23.1-34.2ºC 68.2% THI 78.3 – 81.3 × 39 d	Same subject	• ↓ DMI and ↓ WI and ↓ BW • ↓ rumination and ↓ walking time
Al-Dawood (2017)	Goat	Baladi	Adult (5 yr old)	33.3ºC 35.6% × 35 d	Indoors	• ↓ BW • ↑ circulating leptin and ↑ adinopectin • Circulating NEFA and BHB not affected
Mabjeesh et al. (2013)[5]	Goat	Israeli Saanen	Non-lactating goat	37.0ºC 70.3% 12 h/d × 60 d	Same subject	• ↑ RT • Cofounding effects with photoperiod
Hooper et al. (2018)	Goat	Saanen	Dry goat (5 yr old)	Solar radiation 10 h/d × 3 d	Same subject	• ↑ RT, ↑ RR and ↑ dorsal temperature • ↑ circulating cortisol and ↓ circulating T4 • ↑ mRNA HSP in blood leukocytes
Samara et al. (2016)[5]	Goat	Crossbred	Pubert (6 mo old)	41.9 ºC 6.2% THI 80 × 8 wk	Indoors	• ↑ BW, ↑ ADG, and ↑ FCR in crossbred compared to purebred
Shilja et al. (2016)	Goat	Osmanabadi	Adult buck (1 yr old)	THI 78 6 h/d × 45 d	Indoors	• ↑ Lying time ↑ WI • ↑ RT and ↑ RR • ↑ HSP70
Wang et al. (2016)[5]	Goat	Saanen	Lactating goat	24 – 35ºC 55% 12 h/d × 14 d	Same subject	• DMI not affected • ↑ RT and ↑ RR • ↑ MDA and ↓ TAP
Gaughan and Mader (2009) (exp. 1)[5]	Cattle	Crossbred	Pubert heifers Pubert steers	34.1ºC THI 74 × 9 d	Winter season	• Physiological parameters not affected
Yazdi et al. (2016)[5]	Cattle	Holstein	Bull calves (6-7 mo old)	29.9 – 41.0ºC THI ≥ 85 8 h/d × 9 d	Same subject	• ↓ DMI • ↓ circulating glucose • ↑ circulating insulin and ↑ BUN
Gaughan and Mader (2009) (exp. 3)[5]	Cattle	Angus	Adult steers	25 – 36ºC THI 83 7 h/d × 5 d	Same subject	• ↓ DMI and ↑ WI • ↑ RT and ↑ RR

Table 2.2. (Continued)

Reference	Specie	Breed	Age[1]	HS treatment[2]	CTR group[3]	Main effects
Adin et al. (2009) (exp. 1)[5]	Cattle	Holstein	Dry cow	27.0ºC 78.0% THI 75 – 80 × 8 wk	Cooling group	• ↓ DMI and ↑ WI • BCS and milk (yield, ECM, FCM) not affected • ↓ quantity and quality of colostrum • ↑ disorders • ↓ HSL, ↑ ACC and ↑ FAS activities in mammary tissue • ↓ FS and ↑ ACC activities in SC tissue
Adin et al. (2009) (exp. 2)[5]	Cattle	Holstein	Dry cow	29.5ºC 68.3% THI 78 – 82 × 8 wk	Cooling group	• ↓ DMI • ↓ BCS • ↓ quantity and quality of colostrum • ↓ milk (yield, protein, ECM, FCM)
Tao et al. (2011)[5]	Cattle	Holstein	Dry cow	THI 76.6 × 46 d	Cooling group	• ↓ DMI and ↓ milk (yield, lactose, fat, SCC) • ↓ ADG
Fabris et al. (2019)[5]	Cattle	Holstein	Dry cow (early/last period)	THI > 68 × 45 d	Cooling group	• ↓ DMI and ↓ ADG decreased • ↓ milk lactose and protein
Gao et al. (2017)	Cattle	Holstein	Lactating cow (2nd parity)	32 – 36ºC 40% THI 84.5 × 9 d (×2)	Same subject	• ↓ DMI and ↓ milk yield • ↑ RT and ↑ RR
Kovács et al. (2018)	Cattle	Holstein	Bull calves (1 mo old)	27.8-38.8 ºC × 1 d	Shade	• ↑ Lying down frequency
Haque et al. (2012a)	Buffalo	Murrah	Pubert (1-2 yr old) Adult (3-4 yr old)	40ºC»42ºC»45ºC 50.0% 4 h/d at 3-d interval	Same subject	• ↑ circulating HSP70 with ↑ temperature • ↑ HSP70 young > adult
Haque et al. (2012b)	Buffalo	Murrah	Pubert (1-2 yr old) Adult (3-4 yr old)	40ºC»42ºC»45ºC 50.0% 4 h/d each at 3-d interval	Same subject	• ↑ RT and ↑ RR (young > adult) • ↑ circulating cortisol and NEFA • ↓ circulating insulin and T4 • Circulating T3 young > adult

Legend: ACC acetyl-CoA carboxylase; ADG average daily gain; BCS body condition score; BHB beta-hydroxybutyrate; BUN blood urea nitrogen; BW body weight; CON control group;COX-2 cyclooxygenase-2; d days; DMI dry matter intake; ECM energy corrected milk; ESTR-α estrogen receptor-α; FAS fatty acid synthase; FCM fat corrected milk; FSHR follicle stimulation hormone receptor; GH growth hormone; GHR growth hormone receptor; HR heat rate; HS heat stress; HSL hormone-sensitive lipase; HSP heat-shock protein; Htc hematocrit; IGF-1 insulin-like growth factor-1; LHR luteinizing hormone receptor; MDA malondialdehyde; mo months; NEFA non-esterified fatty acids; PGF2-α prostaglandin F2-α; RR respiration rate; RT rectal temperature; SC subcutaneous; T3 triiodothyronine; T4 thyroxine; TAP total antioxidant potential; THI temperature-humidity index; TRT treatment; WB whole blood; WI water intake; yr years.

[1] Referred to age of animal, productive purpose, or specific production stage.

[2] If available, ambient temperature (ºC), relative humidity (%), temperature-humidity index (THI), hours of exposure per day (h/d), and number of days/weeks/months of exposure (× d, × wk, × mo) are provided. "»" used to indicate consecutive treatment temperatures; time interval in-between is specified.

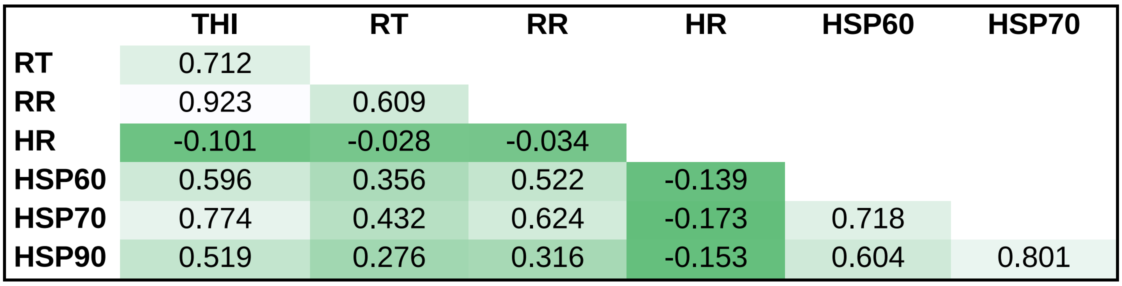
[3] Referred to control group used as reference to compare HS responses (same subject, cooling group, rainy season, indoors, and winter season).

[4] Further differences between subgroups are indicated as follows: "↓ Variable sex 1 > sex 2", as a reduction on "Variable" greater for "sex 1" than "sex 2".

[5] Indicates the evaluation of any other treatment in the study, in addition to heat stress (HS).

In addition, some authors aimed to associate these physiologic indicators to HSP biomarkers of HS. Studies performed with Murrah buffaloes (Haque et al., 2012a) and Barbari goats in India (Dangi et al., 2014, 2016; Yadav et al., 2016), Holstein cows in China (Min et al., 2015), or Saanen goats in Brasil (Hooper et al., 2018) did evidence that increases in RT and RR were observed after a variety of HS challenges (acute, long-term, or progressive heat exposure or solar radiation). The HSP60, 70 and 90 were also upregulated shortly after the stress was introduced. Furthermore, both type of parameters were strongly correlated (Table 2.3).

Table 2.3. Correlation coefficient[1] among physiological parameters, THI, and relative mRNA expression of heat-shock proteins (HSP) (adapted from Dangi et al., 2014).



	THI	RT	RR	HR	HSP60	HSP70
RT	0.712					
RR	0.923	0.609				
HR	-0.101	-0.028	-0.034			
HSP60	0.596	0.356	0.522	-0.139		
HSP70	0.774	0.432	0.624	-0.173	0.718	
HSP90	0.519	0.276	0.316	-0.153	0.604	0.801

Legend: THI temperature-humidity index; RT rectal temperature; RR respiration rate; HR heart rate.

[1] Color indicates the strength of the correlation (■ weakest correlation to □ strongest correlation).

2.1.1.2. Productive outcomes

The impact of heat stress (HS) has been widely studied in dairy animals due to the dramatic reductions in productive outcomes. Actually, animals with high level of production possess higher metabolic rate, which in turn make it difficult to maintain a state of body-thermal balance under hot environments. The HS can reduce milk production as well as milk quality by declines in fat and protein contents, and an increase in somatic cell counts (SCC) in cows (Lambertz et al., 2014; Gao et al. 2017) and goats (Mabjeesh et al., 2013). The HS also impairs the development of the mammary gland during the dry period (Adin et al., 2009; Fabris et al., 2019) and reduces milk production in the subsequent lactation. The HS not only reduces dry matter intake (DMI), but also causes the redirection of the resources towards mechanisms of protection such as using protein to synthesize HSP (Gao et al., 2017).

Furthermore, these deleterious effects caused by HS may affect the entire reproductive performance of females. Reproductive efficiency is altered by HS in terms of degenerative changes in the uterus and downregulation of follicle stimulating hormone (FSHR), luteinizing hormone receptor (LHR), estrogen receptor α (ESTRα), prostaglandin F2α (PGF2α) and cyclooxygenase-2 (COX-2) (Amitha et al., 2019).

With regard to the impact of HS on growth of young animals, a THI > 85 during 8 h/d for 9 d reduced the DMI by 20%, but average daily gain (ADG) was unaffected (Yazdi et al., 2016), whereas adults goats (5 years old) experienced declines in body weight (BW) during summer at 33ºC compared to non-stressed under-shade animals (Al-Dawood, 2017). The reductions in BW have been associated to downregulation in liver of those genes related to growth hormone (GH), GH receptor (GHR), and insulin-like growth factor (IGF-1), accompanied by the presence of degenerative changes in the liver of HS goats at an extreme THI of 87 (Angel et al., 2018).

When assessing growth under HS, breed have to be considered since crossbred goats have shown to be more thermotolerant at maintaining higher BW, ADG and feed conversion ratio (FCR) than those purebred at THI of 80 (Samara et al., 2016).

2.1.1.3. Metabolic changes

One of the most deleterious effects of HS on livestock is the usual decrease in dry matter intake (DMI). Nevertheless, Yazdi et al. (2016) observed that growing calves at THI ≥ 85 for 8 h/d during 9 d had higher concentrations of glucose compared to thermo-neutral period and that their circulating insulin was increased. These results suggested a preferential use of glucose as an energy resource, as an adaptive response to HS, instead of fat mobilization. Similarly, Al-Dawood (2017) observed no differences in blood non-esterified fatty acids (NEFA) or beta-hydroxybutyrate (BHB) concentrations in Baladi goats exposed to 33.3ºC under non-shade treatment compared to 24.4ºC shade treatment during the summer. However, increases in serum adiponectin and leptin were detected; possibly because adiponectin plays a role in glucose uptake and also leptin contributes to the regulation of the appetite. Also, Min et al. (2015) observed greater circulating insulin, leptin and adiponectin concentrations in Holstein cows under HS for three weeks.

However, other authors reported the opposite response with decreasing plasma insulin concentrations and increases of NEFA in both young and adult Murrah buffaloes (Haque et al., 2012b) and the reduction of thyroid hormones concentration in Saanen goats (Hooper et al., 2018). Moreover, in these studies cortisol was observed to rise, as a hormone relevant in the stress response. It has to be considered that exposure time to heat differed among trials, so the later works evaluated the responses at an earlier exposure to HS that could trigger a quickly insulin resistance thereafter changing into the adaptive responses (Morera et al., 2012).

Altogether, it seems that regardless of the genetic background, metabolic shifts might eventually turn into a similar direction. However, there is an interesting meta-analysis done by Salah et al. (2014) evaluating the nutrient requirement of animals in warm climates. In that work, animals representative of tropical and warm areas were shown to have higher maintenance requirements of energy than those from temperate climates.

2.1.1.4. Behavioral response

Behavior is a phenotypical trait that is very sensitive to the environment. One of the first changes that can be observed in animals that are under stressful conditions is a change in their behavior response. Within behavior, the way animals react to novel situations is also influenced by the environmental conditions (Barba-Vidal et al., 2017; Yang et al., 2018). Therefore, behavior is a sensitive measure to investigate changes of perception of the environment.

Regarding HS, acclimatization imposes behavioral changes in the animals that allow them to dissipate the head load and adapt to external conditions (Li et al., 2018). Among behavior measurements, feeding behavior and rumination have been the most studied responses in heat stress trials (Galán et al., 2018; Table 2.2), as indicators of stress and anxiety (Herskin et al., 2004; Bristow and Holmes, 2007). In addition to reduce feeding, lying and drinking are considered as ideal biological markers for assessing the severity of the heat stress response (Shilja et al., 2016; Marchesini et al. 2018), based on the fact that more time spent lying and longer inactivity are usually observed after high temperature exposure as a strategy to dissipate heat through convection (Kaliber et al., 2016; Kovács et al., 2018).

Among the studies listed in Table 2.2, Li et al. (2018) analyzed the behavior of sheep, based on sex differences, within a semi-open barn exposed to extreme heat stress. Differences in the time spent standing and lying were detected according to the level of HS, being decreased and increased, respectively as HS level increased. Furthermore, there were differences between sexes in the rumination and walking, being ewes more active than rams, which suggests greater capacity for ewes to cope with HS

2.1.2. Effects of cold stress on ruminants

Extreme cold environmental temperatures are often considered a challenge to animal health and body homeostasis. Some authors have demonstrated that ruminants exposed to cold environments can cope with stress situations by physiological and behavioral changes. This allows them to maintain their energy homeostasis but results in eventual decreases in performance and production efficiency. Table 2.4 summarizes the main performance, physiological, and metabolic changes of domestic ruminants in response to cold stress.

2.1.2.1. Physiological response

In conditions of low temperatures, ruminants as homeotherms may develop mechanisms to maintain constant body temperature. They reduce the RR (Sano et al., 1992, 1999) in order to decrease the evaporative cooling through the respiratory tract. Also, the metabolic rate increases to produce extra heat as a defense mechanism. Metabolic rate can be measured by heart rate (HR), shivering, and blood glucose concentration (Wentzel et al., 1979; Sano et al., 1999).

Wentzel et al. (1979) assessed temperatures between 0-5ºC in Angora goats and observed that animals, especially adults, were capable of maintaining relatively constant RT during 48 h through these mechanisms, whereas the younger goats decreased the RT faster. Exposure of Suffolk lambs to 0ºC for extended period of time promoted heat production during the first 5 weeks thanks to the greater metabolic rates that might have diverted energy away from tissue growth, although RT was lowered. On the contrary, other authors observed increases in RT immediately after the exposure to relative low temperatures of 2.0 to 4.5ºC (Sano et al. 1999; Li et al., 2000; Verbeek et al., 2012).

2.1.2.2. Productive outcomes

Derived from the physiological and metabolic mechanisms developed to cope with the cold stress, it can be expected that animals will show a reduction in the productivity as a result of the higher maintenance requirements (more energy is used for heat production).

Within this scenario, also lactating animals may suffer from a decline in milk yields up to temperatures as of -10ºC and below (Brouček et al., 1991; Angrecka and Herbut, 2015) and even damage in peripheral tissue such as the udder as a consequence of reduced blood flow to the mammary gland (Thompson and Thomson, 1972) or possibly to freezing (Constable et al., 2016). In contrary to heat stressed animals, under a wide range of cold environments (from -19 to -1ºC), feed intake is usually incremented regardless of age or production stage (Brouček et al., 1991; Gaughan and Mader, 2009) to cover the higher energy requirements under cold temperatures.

Results from the study of Ekpe and Christopherson (2000) demonstrated that young lambs exposed to 0ºC reduced average daily gain (ADG) the first week but presented an increased voluntary feed intake that was maintained over an experimental period of 15 weeks. Animals were eventually capable of resuming gains over the following weeks and revert negative effects in feed efficiency, likely showing a habituation to prolonged cold exposure. Nevertheless, exposure to milder cold conditions (approximately 0.5ºC) for 2 d (Thompson and Thomson, 1972), resulted in no change in DMI despite reduced water consumption. Additionally, sheep exposed to 2ºC experienced no change in feed intake or water consumption (Sano et al., 1999).

Table 2.4. Summary of the main changes observed in domestic ruminants (sheep, goat, and cow) when exposed to cold stress conditions.

Reference	Specie	Breed	Age[1]	CS treatment[2]	CON group[3]	Main effects[4]
Ekpe and Christopherson (2000)[5]	Sheep	Crossbred (Suffolk)	Pubert lamb (6 mo old)	0.0ºC × 15 wk	TN group	• ↑ DMI, ↓ ADG and ↓ FCR during 1st 5 wk • ↓ RT, ↑ heat production during 1st 5 wk
Sano et al. (1992)[5]	Sheep	Suffolk (shorn)	Adult ram (1-2 yr old)	0.0ºC × 2 wk	Same subject	• ↓ RT and ↑ HR • ↓ insulin sensitivity to glucose
Sano et al. (2007)	Sheep	Corriedale × Suffolk	Adult and ewes (2-3 yr old)	2 ± 1°C × 19 d	Same subject	• ↑ circulating NEFA and ↑ insulin sensitivity to glucose • No changes in feed intake
Verbeek et al. (2012)[5]	Sheep	Coopworth × Texel	Pregnant ewe (4-5 yr old)	4.5ºC × 6 h	Same subject	• ↑ vaginal temperature and ↓ skin temperature • Circulating insulin ↑ in good BCS and ↓ in poor BCS • Circulating leptin ↓ in good BCS and in poor BCS not affected • ↑ circulating T4 and T4 poor BCS < good BCS at recovery • ↑ circulating cortisol and ↑ circulating glucose, BHB and FFA
Wentzel et al. (1979)	Goat	Angora	Young barren Pregnant goat	0.0 – 5.0ºC × 48 h	TN group	• ↑ HR and ↑ shivering, RT constant in adults • Collapse in young > adult, RT drops to 34.0ºC • Circulating glucose peaks after 4h and drops within 20 h • ↑ circulating corticosteroids and tyrosine
Bøe et al. (2007)[5]	Goat	Norwegian	Non-lactating pregnant goat (> 1.5 yr old)	-12.0 – 8.0ºC × 19 d	Moderate-temperature group	• ↑ circulating FFA 1st day • ↓ lying time and ↑ active and ↑ feeding
Kour et al. (2014)[5]	Goat	Marwari	Non-lactating goat (5 mo-4 yr old)	6.8ºC × 2 mo	Same subject	• ↓ circulating TSP male < female, older < younger • ↑ circulating TFA female > male and older > younger
Kour et al. (2017)[5]	Goat	Beetal Toggenbourg	Adult female	11.4ºC 79.5% THI 53.2 × 2 mo	Summer season (also, autumn)	• ↑ circulating glucose and cholesterol • ↓ circulating TP and albumin and BUN • Values in Toggenberg > Beetal breed
Faulkner et al. (1980)	Goat	British Saanen	Lactating goat (4-10 yr old)	-0.5 – 0.5ºC × 24 h	TN group	• ↑ circulating glucose and ↓ glucose uptake by the udder • ↑ circulating corticosteroids
Gaughan and Mader (2009) (exp. 2)[5]	Cattle	Crossbred	Pubert steer	-4.9 – 2.9ºC THI 31.3 × 9 d	Summer season	• ↑ DMI, decreased WI, influence of diet.
Kang et al. (2016)	Cattle	Korean	Pubert steer (10 mo old)	1.0ºC × 4 wk	Same subject	• ↓ DMI, ↓ ADG and ↑ FCR • ↑ circulating NEFA and cortisol not affected • ↑ complement compound and ↑ CD4+ and CD8+ T-cells

Table 2.4. (Continued)

Reference	Specie	Breed	Age[1]	CS treatment[2]	CTR group[3]	Main effects[4]
Brouček et al. (1991)	Cattle	Slovak Spotted × Holstein-Friesian	Lactating heifer (2-3 yr old)	-10.2 – -4.3ºC × 60 d	Same subject	• ↑ DMI and ↓ milk yield • ↑ circulating glucose and FFA and ↓ sodium
Angrecka and Herbut (2015)[5]	Cattle	Holstein-Friesian	Lactating cow	-12.3 – -7ºC × 7 d	Same subject	• Correlation between milk yield and combination of air temperature and air velocity

Legend: ADG average daily gain; BCS body condition score; BHB beta-hydroxybutyrate; BUN blood urea nitrogen; BW body weight; CON control group; CS cold stress; d days; DMI dry matter intake; FCR feed-conversion ratio; FFA free fatty acids; HR heart rate; mo months; RT rectal temperature; T4 thyroxine; TFA total fatty acids; TN thermos-neutral; TP total protein; TSP total serum protein; wk weeks; yr years.

[1] Referred to age of animal, productive purpose, or specific production stage.

[2] If available, ambient temperature (ºC), relative humidity (%), temperature-humidity index (THI), hours of exposure per day (h/d), and number of days/weeks/months of exposure (× d, × wk, × mo) are provided. "»" used to indicate consecutive treatment temperatures; the time interval in-between is specified.

[3] Referred to control group used as reference to compare CS responses (same subject, TN group, temperature-moderate group, and summer season).

[4] Further differences between subgroups is indicated as follows: "↓ Variable breed 1 > breed 2", as a reduction on "Variable" greater for "breed 1" than "breed 2".

[5] Indicates the evaluation of any other treatment in the study, in addition to cold stress (CS).

2.1.2.3. Metabolism changes

Contrary to heat-stressed animals, where body fat reserves are not mobilized, cold-stressed ruminants have shown to maintain high levels of lipolytic activity. Hence, insulin, as an antilipolytic hormone, is observed to be low under low temperatures (Sanz Sampelayo et al., 2000), but also to be less responsive to glucose, while on the counterpart tissues remain more sensitive to insulin (Sano et al., 1992).

Consequently, circulating glucose has been observed to rise in young and adult animals regardless of cold exposure time (hours or a season) and severity (from approximately -19 to 11.4 ºC) (Wentzel et al., 1979; Brouček et al., 1991; Kour et al., 2017), and also did lipid metabolites such as NEFA and cholesterol (Bøe et al., 2007; Kang et al., 2016; Kour et al., 2017). Moreover, a decrease has been observed reduced the percentages of glucose extraction by the udder in dairy goats (Faulkner et al., 1980), suggesting that this factor is responsible for the reduction of milk secretion during cold exposure.

On the contrary, the study of Ekpe and Christopherson (2000) found that young lambs under 0ºC for 15 weeks presented higher insulin concentrations. Other inconsistencies are seen with cortisol level responses. In the same work cortisol was observed to increment at the early and late experimental periods. This event might be a possible mechanism to promote the glucose and fatty acid turnover, resulting in supplying energy for the thermogenesis and for demands in substrate mobilization. Increases in corticosteroids were also observed in young Angora goats after only 48 h of cold exposure (Wentzel et al., 1979). However, as reviewed by Storbeck et al. (2009), this response is not necessarily always given properly, and other factors such as body condition can limit metabolic response as a mechanism of adaptation to save energy (Verbeek et al., 2012). In the same line, differences in mobilization of sources may depend on the sex of animals and on the capacity acquired with age (Kour et al., 2014).

2.1.2.4. Behavioral response

Scarce reports have evaluated the behavioral response by recording activity (feeding, rumination, and other activities) or posture. To our knowledge, only the work of Bøe et al. (2007) quantified these parameters in dry dairy goats and observed that they responded to low temperatures by decreasing the lying time and increasing the time spent active and eating.

This way, animals produce heat, which is the opposite of what happens during HS as previously indicated.

Shivering, as a thermoregulatory response for heat generation, is another response to cold temperatures (Wentzel et al., 1979). However, during severe hypothermia, shivering may be due to the accumulation of metabolites and rigidity of muscles (Khounsy et al., 2012). Another variable to measure might be coat condition (that should be dry and clean) in terms of apparent density (Tarr, 2010), or presenting piloerection (Lenis Sanin et al., 2016), which may enlarge the surface covered by hair and reduce the loss of heat. Furthermore, other measurable behaviors developed to combat low temperatures can take advantage of biological mechanisms, especially in sheep. These include huddling when feeling cold due to their gregarious nature, or seeking for shelter (Lenis Sanin et al., 2016), although experimental conditions may hinder their assessment.

2.2. Fetal programming

Fetal programming consists of the impact that has maternal environment during the prenatal development, i.e. during the embryonic and/or fetal stages, on the early and adult life of the offspring in terms of growth, disease (Funston et al., 2010), as well as behavior (McGowan and Matthews, 2018). So, it is suggested that an impaired growth of the fetus during the gestation is correlated with the development of chronic diseases in the later life (Vo and Hardy, 2012).

In the early publications of Dutt (1963) and Everitt (1964), there were presented the first insights exploring the regulation of the offspring outcomes during the pregnancy by exposing ewes to 32.2ºC the first days post-mating or to undernutrition programs in the early gestation. Nonetheless, fetal programming *per se* initially departed from human epidemiological data whereby low birth weights of infants were associated with poor maternal nutrition, leading to the rise of diseases during the adulthood, such as coronary heart diseases or diabetes. Studies evaluating fetal programming in livestock are generally more recent (Du et al., 2010).

There are several insults or treatments during the prenatal period, including variety of conditionings: stress type, severity, and time when applied during the pregnancy. The most interesting scenarios with implications in the productivity of cows, sheep and goats, may include thermal stress (heat or cold), and nutrition level.

2.2.1. Fundamentals and mechanisms

Beyond the genetic contribution of both parents to the development of the offspring, the intrauterine growth retardation (IUGR), which is the impaired growth and development of the embryo and/or the fetus during the pregnancy (Wu et al., 2006), can be also conducted by the environmental factors aforementioned (Figure 2.2). Measurement of IUGR animals is an easy practice on farms and clinics, because the weight of the fetus or the weight at birth relative to the gestational age is a criterion for its determination (Wu et al., 2006).

IUGR can be a natural mechanism to protect the dam in adverse situations such as cases of undernutrition or multiple fetus pregnancies, but it is not necessarily beneficial for the progeny or for the efficiency of the livestock production. So far, however, most of the studies using IUGR models have been focused on human-based models such as rodents and sheep, inducing the IUGR by modifying the nutrition program or by changing environmental temperatures (Morrison, 2008). Also, stressful insults on the mother such as inducing depression (Schroeder et al., 2012), alcoholism (Schneider et al., 2002) or the direct exposure to glucocorticoids (Dodic et al., 2002), as an old practice to aid the lung maturation of infants at risk of preterm delivery, have been implemented.

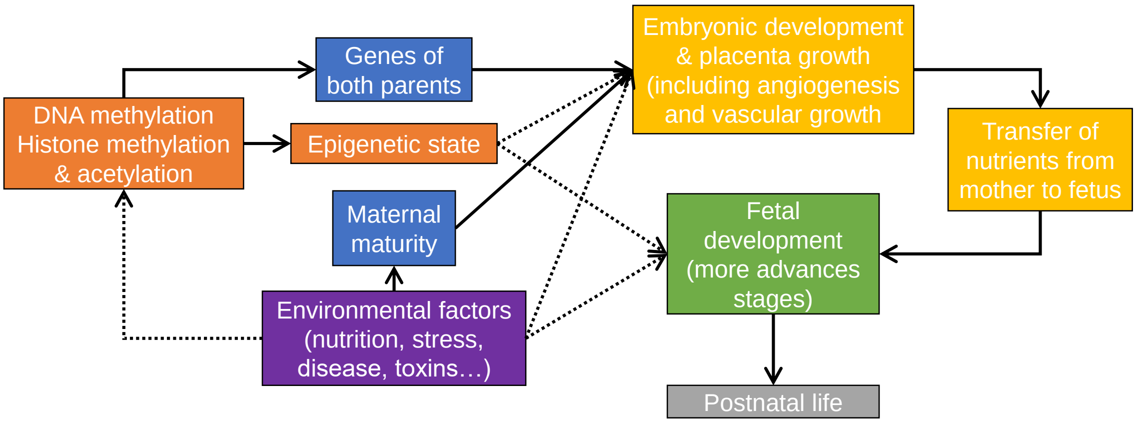


Figure 2.2. Regulation of fetal development, basics for IUGR models (adapted from Wu et al., 2006). Legend: → causative relations between prenatal and other prenatal or postnatal development; ⇢ main causative factors reviewed in the frame of the present Thesis (epigenetic mechanisms and environmental conditions).

Placental insufficiency (PI) is a cause of fetal malnutrition that manifests into IUGR (PI-IUGR) (Yates et al., 2011). With PI-IUGR models of fetal programming, an expected poor placental development may lead to suboptimal substrate supply to the fetus growth (Morrison, 2008). The changes observed in fetal and neonatal life of offspring in terms of physiology, metabolism and consequent growth using IUGR models are summarized in Table 2.5.

Briefly, during the fetal life, the PI will reduce the provision of O_2, glucose and amino acids to the fetus during gestation (Limesand et al., 2007). Catecholamines, especially epinephrine, increase in response to hypoxemia, which suppress insulin secretion to maintain glucose levels (Chen et al., 2010), favoring insulin-independent neural tissue detrimental to liver, skeletal muscle and adipose tissues (Limesand et al., 2007). In turn, skeletal muscle enhances insulin sensitivity to restore the uptake of glucose for anaerobic metabolism and the production of lactate, a substrate used to restore the gluconeogenesis in the liver, and hence increasing the hepatic production of glucose by the liver in the fetus (Limesand et al., 2007). This way oxidative energy production relies on the mobilization of fat induced by the catecholamines

(Chen et al., 2010). In parallel, hypoglycemia can stimulate cortisol secretion from the adrenal cortex (Yates et al., 2011), if not transferred by the mother (Coulon et al., 2015). At postnatal life, the long exposure to catecholamines will eventually desensitize the β2-adrenergic receptors of the adipose tissue, what will enhance the adiposity in the IUGR lambs (Chen et al., 2010). This, in turn, results in a higher sensitivity by the pancreatic β-cells to glucose, causing more secretion of insulin in the neonatal lamb as well as greater uptake of glucose by the skeletal muscles (Chen et al., 2010). Eventually, the postnatal growth will show a catch-up event and an increase of fat deposition.

Table 2.5. Responses of embryo/fetus and postnatal offspring to prenatal stress based on an ovine IUGR model induced by hyperthermia (information depicted from Yates et al., 2011).

	Embryo/fetal life	**Neonatal life**
Physiology	• Reduction of O_2 and glucose	
Metabolism	• Increase of catecholamine secretion from adrenal medulla • Insulin secretion reduced and increased glucagon secretion • Stimulation of cortisol secretion • Skeletal muscle increases sensitivity to insulin • Lactate production by anaerobiosis • Oxidative energy by amino acids and mobilization of fat	• β2-adrenergic receptors desensitization on adipose tissue • Hypersensitivity to glucose and oversecretion of insulin • Skeletal muscle more sensitive to insulin
Growth	• Reduction of fetal mass	• Catch-up growth • Adiposity increases

For sheep, the study of functional and structural changes of placenta and the relationship between maternal-fetal nutrient transfer and metabolic changes is relatively recent (Hansen, 2015; Grigsby, 2016). In fact, PI-IUGR sheep models can be obtained by removal of the endometrial caruncles prior to conception (De Blasio et al., 2007), by maternal nutritional modifications in the pregnant ewe (Rae et al., 2002; Sinclair et al., 2007a; Jaquiery et al., 2012), by injection of synthetic glucocorticoids (Dodic et al., 2002) as well as by experimental induction of hyperthermia (Limesand et al., 2007; Chen et al., 2010). Beyond sheep as a model mainly for human IUGR, calves born to cows exposed to heat stress (removal of non-cooling systems) during the dry period (late pregnancy) showed to some extent parallel responses on intrauterine growth and postnatal performance, with increased sensitivity to insulin, although with no compensatory growth afterwards nor higher sensitivity of pancreas to glucose (Tao et al., 2014).

2.2.1.1. Epigenetic mechanisms during fetal programming

In this section, the molecular events that permanently may control the mechanisms underlying the fetal programming will be presented. Some of these adaptations in early development are probably mediated, at least partially, by changes in the epigenetic mechanisms (Palma-Gudiel et al., 2015). Epigenetic mechanisms modulate the patterns of the expression of genes without altering the genetic sequence. The principal epigenetic mechanisms are listed and described on Table 2.6.

Epigenetic modifications represent a novel and hot area of research for animal science. However, most research has been focused on human research, and the most common animal-based model was rodents. The review of (Palma-Gudiel et al., 2015) addressed all the literature available regarding the promoter of the *NR3C1* gene, which includes a CpG island that has been a target for the study of methylation patterns. The importance of the *NR3C1* gene relies on that codes for glucocorticoid receptor (GR), involved in the hypothalamus-pituitary-adrenal (HPA) axis function. This is associated with modifications in the offspring after suffering prenatal stress (McGowan and Matthews, 2018) that can later affect to stress disorders in the fetal development, early life, puberty and adult life (Palma-Gudiel et al., 2015). Palma-Gudiel et al. (2015) reported that most of the published works correlated early life stress with DNA methylation patterns in the CpG island of *NR3C1*. Despite the divergent results, as a general remark observed hypermethylated patterns with prenatal/early stress in life may be associated with downregulation of GR expression, basically in the brain, with discrepancies between sexes. Although there has been published a diversity of reviews claiming for the potential of epigenetics on domestic ruminants for the last ten years, with especial mention to nutrition (Nuyt and Szyf, 2007; Singh et al., 2012; Funston and Summers, 2013; Chavatte-Palmer et al., 2018), scarce works have focused on the epigenetic mechanisms influencing the fetal development.

Sinclair et al. (2007b) were the first authors that designed a diet lacking methyl donors given to sheep from week 8 before until 6 d after conception. This diet was deficient in cobalt and sulphur to diminish the synthesis of sulphated amino acids and vitamin B_{12} by ruminal microorganisms, expecting to reduce DNA methylation in the embryos that were transferred afterwards to control females to evaluate the direct effect in the embryos. During gestation (at 90 d), the authors observed that 4% of the 1400 CpG islands, dispersed over the genome in the fetal liver, showed differential patterns, affecting to a larger extent males, all associated with the increased adiposity, insulin resistance and altered immune function in the adult offspring.

Table 2.6. Summary of the main epigenetic mechanisms (depicted from Wu et al. (2006), and Vo and Hardy, 2012).

DNA methylation	Mechanism	• Direct methylation of CpG sites[1] within the DNA by methyltransferases through typical methyl donors, such as methionine, choline or folic acid.
	Implication	• Methylation is expected to impair the initiation of the elongation or termination of a gene, and therefore silence the gene.
Post-translational histone modification	Mechanism	• Histone can be modified at post-translational level by methylation, acetylation, phosphorylation, ubiquitination and DP-ribosylation by a range of histone acetylases and methyltransferases, among other enzymes.
	Implications	• Depending on the added radical, for the depression of expression of a gene, methylation of histones will be enhanced while acetylation reduced.
microRNA (miRNA)	Mechanism	• Consists of short non-coding RNA molecules of 20-25 nucleotides that regulate gene expression by degrading mRNA and/or repressing the translation, naturally participating in life cycle of cells. They only need a partial sequence homology to bind to 3'-untranslated region to induce the cleavage of RNA.
	Implications	• miRNA repress productive translation, so silence gene expression.
Others	Nuclear factors (NF)	• Consist of transcription factors that are mediated by ligands such as hormones, vitamins, and participate in the regulation of gene transcription. Glucocorticoid receptor and mineralocorticoid receptor are commonly studied since they are involved in the fetal hypothalamus-pituitary-adrenal axis and the corresponding ligand is cortisol.

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[1] A CpG site or island consists of high-frequency cytosine (C) and guanine (G) region (segment of at least 200 base pairs with C and G contents of ≥50%) linked by a phosphodiester bond, referred as "p", between the adjacent nucleotides. This bond differs from the hydrogen bond existing between C and G across the two strands from the DNA helix. CpG islands occur more frequently in and around the promoter region of genes, entailing an important role for gene prediction since DNA methylation occurs to C nucleotides (Kakumani et al., 2012).

Later, the work of Stevens et al. (2010) also demonstrated that mature ewes undernourished at different moments, from 60 d premating to 30 d postmating or separately before (-60 d) or after (+30 d) mating, experienced changes in the promoter of the *POMC* gene (coding for proopiomelanocortin, which is the precursor of the ACTH) and the *GR* gene in the fetal hypothalamus. They observed that the dietary treatment was associated with higher H3K9Ac (acetylated histone H3K9) and hypomethylation of *POMC* and *GR* in the hypothalamus, and also that *GR* was more expressed, especially when stressing ewes over the entire period (-60 to +30 d relative to mating). However, they did not evaluate postnatal life.

More recently, Skibiel et al. (2018) evidenced for the first time the linkage between maternal thermal stress in cows and the epigenome response from the offspring. The methylation patterns in the neonatal liver of calves and later in the adult mammary gland during the first lactation, born to heat stressed or cooled dams, were measured by double restriction enzyme - bisulphite sequencing (RRBS) and were compared. Bulls showed differential patterns of methylation on CpG islands across the genome, but no differences were detected across the mammary tissue of mature females born to heat-stressed or cooled dams. Therefore, calves that were exposed to heat stress *in utero* exhibited differential methylation patterns, especially at birth and to a lesser extent at maturity. Nonetheless, authors were not able to find correlations between the methylation degree and gene expression.

Most evidences of the deleterious effects of stress, including heat stress, during the prenatal life on the offspring have been obtained using sheep during the periconceptional period (considered as the most critical period). However, the specific moment at which the insult is implemented may encompass different embryonic and fetal stages, and thus potentially affecting specific organs or systems. Therefore, the following section discusses the potential window in the prenatal life during which different environmental factors can exert their epigenetic effects on offspring.

2.2.2. Window of action during fetal programming

The epigenetic effect of a specific environmental factor (e.g. stress) depends on the moment of the pregnancy when the stressful event takes place, its duration, and the postnatal moment when offspring response is evaluated. During the preimplantation growth, the pregnancy hormone progesterone stimulates and maintains the endometrial functions for the growth of the conceptus[1], but also afterwards during the implantation and the placentation. In ruminants, the IFN-τ is the recognition signal by the conceptus to inhibit the development of the luteolytic mechanisms, but it also induces the secretion of prostaglandins and cortisol in early pregnancy to regulate the endometrial functions and the conceptus elongation (Brooks et al., 2014). Implantation and placentation take place afterwards at 14-18 d post-conception in sheep, 17-35 d in cattle, and 15-18 d in goats (McGeady et al.. 2017).

Studies implementing IUGR models in sheep (hyperthermia, caruncolectomy, synthetic glucocorticoids) were designed for early alteration of the fetal placentation and development, with consequences in the postnatal life of offspring as depicted in Figure 2.3. Maximal placental growth, differentiation, and vascularization occur during the early phase of fetal development. The placenta is the major regulator of fetal growth, and it appears that maternal input may affect the development and function of the placenta (Funston et al., 2010). The majority of organs are formed simultaneously with placental development during early gestation, such as kidneys or pancreas (McGeady et al., 2017). Similarly, at this stage, nutrient restriction of the mothers causes deleterious effects on placental development and fetal growth (Zhu et al., 2007).

Other processes such as muscle development take place during embryonic and fetal stages, although most of the growth and maturation takes later during the mid-late gestation. Similarly, adipogenesis takes place at approximately d 105 of gestation in sheep and at d 210 of gestation in cattle, with the miogenesis occurring little earlier than adipogenesis (Du et al., 2010; McGeady et al., 2017). Ford et al. (2007) observed that restricted nutrition during early-mid gestation led to reductions of skeletal muscle mass. On the other hand, the nervous system develops towards the end of gestation (Matthews et al., 2002), which has led to hypothesize that offspring behavior might be altered when mothers exposed to prenatal stressful events during late gestation (Coulon et al., 2015). In fact, Roussel et al. (2005)

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[1] Term used to define the association between the embryo-fetus and extraembryonic membranes. *Conceptus* term begins in the uterus after the hatching of the blastocyst from the zona pellucida, when the blastocyst will grow into a tubular or ovoid form, known as *conceptus elongation*. This process involves exponential growth of the trophoblast and gastrulation of embryo, and onset of extraembryonic membrane differentiation into the yolk sac and allantois (Brooks et al., 2014).

evidenced altered adaptive behaviors and cognitive abilities of offspring born to ewes and goats under stressful managements during late gestation.

Most studies handling prenatal heat stress have been limited to late gestation in dairy cows during the dry off period (typically 2 months before calving) (Figure 2.3). Traditionally, as a non-productive period, good management during this period is often disregarded. More recently, the group of Tao and Dahl (2013) revealed the importance of this period for lactating cows, because of several reasons: (i) approximately 60% of the fetus growth occurs during the last two months of gestation, (ii) at this moment the correct development and growth of the mammary gland is critical for the subsequent milk production, and (iii) cows are under negative energy balance and a transitional immune dysfunction related to more incidences of health and metabolic disorders. Furthermore, cortisol, at least in sheep, can easily cross the placenta barrier during the last third of the pregnancy (Roussel et al., 2005), so the fetal brain may be more susceptible to any distress at this moment.

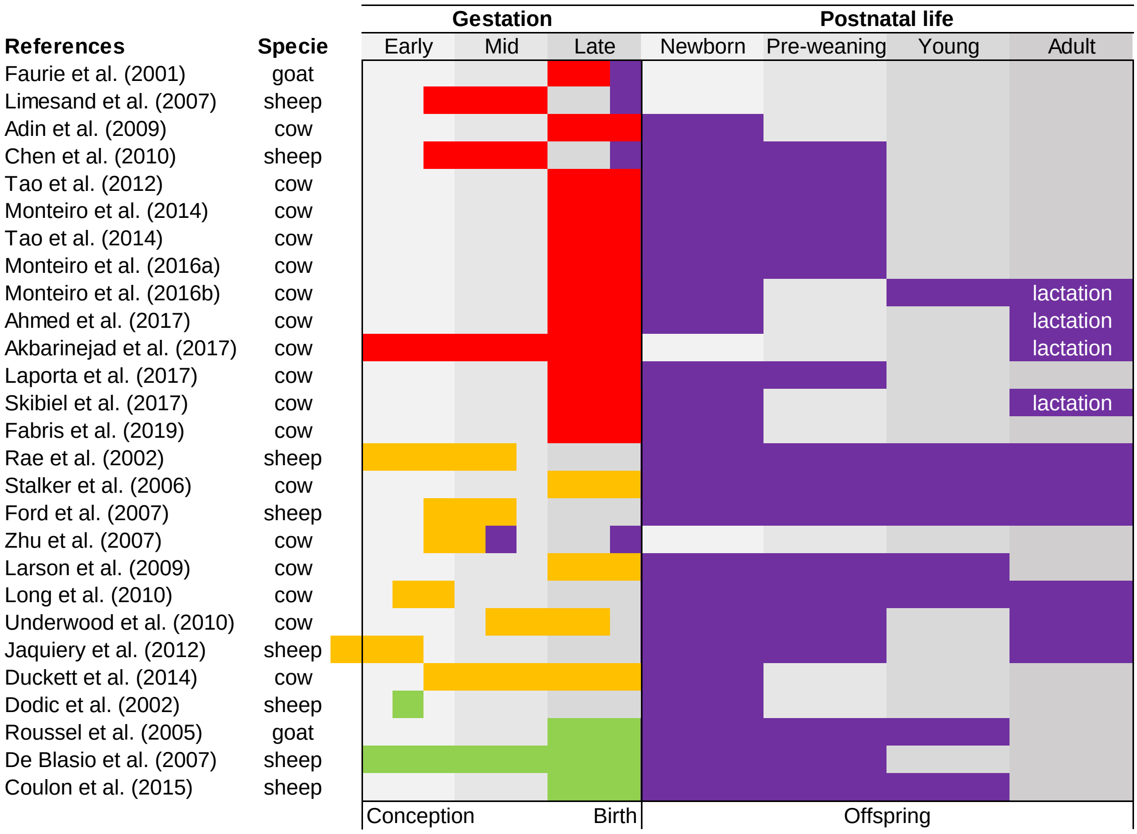


Figure 2.3. Timing of stressful events during pregnancy in common domestic ruminants (goat, sheep, cow) and age of offspring when displaying main altered responses.

Legend: 🟥 heat stress treatment; 🟨 nutrition restriction 🟩 other stress, inducing synthetic glucocorticoid injection, management caruncolectomy; and 🟪 period of evaluating the effects during the postnatal life of offspring, if specified during lactation.

2.2.3. Prenatal heat stress effects

One of the earliest studies evaluating the effects of HS during pregnancy was the study of Dutt (1963). They reported that HS during the first 5 days of pregnancy in sheep increased embryo mortality. The area of prenatal HS has become of interest throughout the last few years, and Table 2.7 summarizes the main findings in calves, lambs, and kids. In addition to the effects during the postnatal life, some results during the fetal life are also presented. In general, offspring born to HS mothers may show impaired postnatal growth, altered metabolism and immune function, and even modifications in the behavior.

Regarding small ruminants, the only work testing prenatal HS in goats demonstrated that the fetus cannot thermoregulate *per se* but depends on the heat production and transfer from the dam. In this case the authors (Faurie et al., 2001) measured fetal and maternal temperatures at late gestation, and the fetus showed a mean temperature 0.6ºC higher, a difference that was narrowed after a HS challenge.

In case of ovine, the IUGR model was most commonly implemented by inducing hyperthermia (40ºC for 12 h and 35ºC for 12 with 30-40% relative humidity) at the early-mid gestation (Morrison, 2008). Thermal-induced IUGR reveals the causes of fetal retarded growth. There is an impaired protein accretion due to the lower levels of glucose and oxygen in addition to the compromised insulin production and the establishment of lactate production at the expense of growth. Later, a catch-up compensatory growth in the early postnatal life is observed with a high stimulation of insulin to glucose and a lower lipolytic response to adrenergic stimulation, thus favoring the accumulation of adipose tissue (Limesand et al., 2007; Chen et al., 2010).

In cows, most of the studies evaluated the effects of HS during the dry-off period (last 2 months of gestation) as shown in Table 2.7. The model was comparing the effects of non-cooling vs. cooling during the dry-off period. The HS in the late gestation may shorten the gestation length and deliver lighter animals at birth. Lower body weight at birth can be explained by shorter pregnancy length and lower feed intake of non-cooled dams. However, Tao et al. (2014) observed reduced birth weight of calves born to non-cooled dams without any differences in gestation length. A direct effect of HS on fetus growth cannot be ruled out (Tao and Dahl, 2013).

Table 2.7. Main effects induced by prenatal heat stress at parturition of domestic ruminant (sheep, goat, cow) and offspring during the fetal and/or postnatal life.

Reference	Specie	Breed	HS conditions[1]	HS time	Parturition	Offspring
Faurie et al. (2001)	Goat	Boer	40ºC 60.0% × 2 h » 6ºC 90.0% × 2 h at 1-wk interval	117 - 150 dG	• ↑↓	• ↑ fetal body temperature (+0.6ºC) dependent on maternal • ↓ △body temperature in HS and ↑ △body temperature in CS
Limesand et al. (2007)	Sheep	Cheviot	40.0ºC:35.0ºC 12 h:12 h 35%	39 - 96 dG		• ↓ placenta weight • ↓ plasma rate and ↓ umbilical cord blood flow • ↓ fetal blood O_2, ↓ glycemia and ↓ circulating insulin • ↑ mRNA GLUT1 in brain and ↑ mRNA glycogenic enzymes in fetal liver
Chen et al. (2010)	Sheep	Columbia-Rambouillet	40.0ºC:35.0ºC 12 h:12 h 35%	40 - 85 dG	• ↓ 3 dG	• ↓ placenta weight and ↓ fetal BW and ↓ birth BW • ↓ mRNA β2-AR and ↓ β2-AR protein in fetal and postnatal perirenal adipose tissue • ↓ circulating NEFA response to EC in lambs
Adin et al. (2009) (exp. 1)	Cow	Holstein	27.0ºC 78.0% THI 75-89 with/out direct cooling	Last 56 dG	• ↓ colostrum quantity and quality	• Birth BW not affected
Adin et al. (2009) (exp. 2)	Cow	Holstein	29.5ºC 68.4% THI 78-82 with/out direct cooling	Last 56 dG	• ↓ dG • ↓ colostrum quantity and quality	• ↓ birth BW
Tao et al. (2012)	Cow	Holstein	THI 78.3 with/out direct cooling	Last 45 dG	• ↓ 4 dG • Colostrum IgG not affected.	• ↓ birth BW, ↓ weaning BW and 7-mo-old BW not affected • ↓ IgG absorption, ↓ PBMC proliferation but IgG stimulation not affected in heifers
Monteiro et al. (2014) (exp. 1)	Cow	Holstein	THI 78.3 with/out direct cooling	Last 46 dG	• ↓ dG	• ↓ birth BW and ↓ pre-weaning BW at birth in calves • ↑ % neutrophil,↓ % lymphocyte at pre-weaning and ↓ WBC proliferation in heifers • ↓ IgG absorption but IgG stimulation not affected in heifers
Monteiro et al. (2014) (exp. 2)	Cow	Holstein	Colostrum from exp. 1 given to calves born to TN-cows	Last 46 dG	• ↑ IgG in pooled colostrum	• ↑ RT in calves born to non-cooled cows. • ↑ IgG stimulation at pre-weaning and weaning • Disease score not affected
Tao et al. (2014)	Cow	Holstein	THI 75 with/out direct cooling	Last 45 dG	• dG not affected	• ↓ birth BW but postnatal BW not affected • ↓ circulating glucose and ↓ glucose response to insulin • NEFA non responsive to insulin

Table 2.7. (Continued)

Authors	Specie	Breed	HS conditions¹	HS time	Parturition	Offspring
Monteiro et al. (2016a)	Cow	Holstein	THI 77 with/out direct cooling	Last 45 dG	• dG not affected	• ↓ weaning BW and ↓ weaning ADG and ↑ pre-weaning DMI • ↓ pre-weaning circulating glucose and ↑ NEFA and ↑ BHB • ↑ glucose clearance and ↓ insulin clearance in IC • NEFA non responsive to epinephrine nor glucose
Ahmed et al. (2017)	Cow	Holstein	THI > 72 × 48 h with/out direct cooling	Last 46 dG	•	• ↓ afternoon RT and ↓ sweating rates in lactating cows
Laporta et al. (2017)	Cow	Holstein	THI > 72 with/out direct cooling	Last 46 dG	• ↓ 4 dG • ↑ colostrum IgG	• ↓ pre-weaning BW and ↓ IGF-1 in calves • ↑ birth and pre-weaning RT • ↓ circulating IgG and ↓ IgG absorption efficiency • Worsened fecal scores and ↓ time standing
Skibiel et al. (2017)	Cow	?	THI > 70 with shade with/out direct cooling	Last 46 dG	•	• ↓ hepatic cells in born bulls • ↑ size of mammary gland alveoli in first-lactation heifers larger • △methylation patterns in liver between bulls born to HS and cooled cows > mammary tissue
Fabris et al. (2019)	Cow	Holstein	THI > 76 with shade with/out direct cooling	Last 45 dG (3-wk periods)	• ↓ dG	• ↓ birth BW at any 3-wk period

Legend: △ differential; HS heat stress; ADG average daily gain; BHB beta-hydroxybutyrate; BW body weight; CS cold stress; dG days of gestation; DMI dry matter intake; EC epinephrine challenge; GLUT1 glucose transporter 1; IC insulin challenge; IGF-1 insulin-like growth factor-1; mo months; NEFA non-esterified fatty acids; PBMC peripheral blood mononuclear cell; RT rectal temperature; THI temperature-humidity index; TN thermos-neutral; WBC whole blood cell; wk weeks; β2-AR β2-adrenergic receptor.

[1] If available, ambient temperature (ºC), relative humidity (%), temperature-humidity index (THI), hours of exposure per time (h) are provided. To indicate consecutive treatment temperatures, "»" is indicated, and the interval of time between each is specified. Control treatments are included in the same cell.

Additionally, calves born to HS mothers experienced greater insulin response to glucose administration (Tao et al., 2014). Monteiro et al. (2016a) later reported that glucose might be the preferred source of energy of pre-weaning calves (from d 8 to d 57 of age) in view of the of lower basal circulating glucose, although offspring born to HS mothers presented insulin resistance in peripheral tissues, especially within the days postpartum (within 8 d of age). Authors hypothesized that offspring's postnatal insulin-independent glucose absorption and basal glucose uptake would have been improved, and that initial insulin resistance might disappear as animals grow.

Tao et al. (2012) also assessed the immune function and growth of calves born to HS cows. Calves showed compromised passive immunity transfer trough decreased apparent capacity of IgG absorption as well as possibly compromised lymphocyte production, although the humoral response was unaffected. Similarly, prenatal HS calves experience lower percentages of lymphocytes and greater neutrophils as well as a reduction in white blood cells proliferation (Monteiro et al., 2014), possibly related to some extent to increases in cortisol (an immunosuppressant) during the first few days postpartum. The impaired passive immunity transfer does not seem to be related to colostrum quality, since Laporta et al. (2017) found that colostrum from HS dams had more IgG contents, but this increased IgG did not result in higher circulating levels of IgG in their calves.

Ahmed et al. (2017) evaluated whether calves that experience HS in utero (last 2 month of gestation) have altered thermoregulatory responses to acute heat stress later in life. Therefore, they exposed calves to a heat challenge consisting of transferring from a barn with shade and evaporative cooling to one with shade but no additional cooling for 48 h. Calves that experienced HS in utero possess an increased capacity to dissipate heat to maintain core body temperature (Ahmed et al., 2017).

In the comprehensive study of Monteiro et al. (2016b), the authors assessed the effect of HS or cooling in late gestation dairy cows during five consecutive summers on the survival and milk production in the first lactation of their calves. A greater percentage of cooled heifers reached first lactation compared with heifers born to non-cooled dams. Additionally, heifers born to non-cooled cows produced 16% less milk up to 35 weeks of the first lactation with no differences in milk fat or protein contents.

In a retrospective study, Akbarinejad et al. (2017) studied the impact of prenatal HS applied during early, mid or late gestation on the reproduction performance of the offspring throughout the first to fourth lactation. The HS delayed the first insemination postpartum, reduced the fertility, prolonged the calving to conception interval and increased the culling rate in the offspring throughout successive lactations. The impact of HS was more marked during the second and third trimesters of gestation.

2.2.4. Prenatal undernutrition

Although the current Thesis does not handle undernutrition during the prenatal period, HS typically results in reduced feed intake of dams, which means lower nutrients (major and minor) provided to the embryo or fetus. It is important to highlight that the effects of nutrient restriction during pregnancy depend on the stage of pregnancy, level, and duration of nutrient restriction as well as the type of nutrient restriction (energy, protein, etc.). Fetal responses to changes in maternal nutrition may be beneficial for the fetus, but the long-term effects of these adaptations might be detrimental if nutrition in the postnatal life does not match that predicted by the fetus prenatally (Cleal et al., 2007). As shown in Figure 2.3, nutrition restriction at early stages of gestation (first third) impacted offspring performance in sheep (Ford et al., 2007; Jaquiery et al., 2012; Rae et al., 2002) and cows (Long et al., 2010).

Long et al. (2010) applied low nutritional levels (55% of those recommended by the NRC) to cows from 32 to 115 d of gestation to study the postnatal growth and development of the offspring. Gestation length was shortened by 4 d with no effect on birth weight or growth rates. Nonetheless, lung and trachea weighed less at slaughter in low-nutrition diets and presented larger muscle fiber areas.

Cleal et al. (2007) fed sheep 100 or 50% of their nutrient requirements between d 1 and 31 of gestation, and then 100% until the end of pregnancy. After lambing, offspring received adequate postnatal nutrition (i.e., mismatched for those lambs exposed to under nutrition during the first 31 d of gestation). These mismatched lambs showed greater pre-weaning growth and exhibited cardiac hypertrophy as well as altered cardiovascular function in adulthood. Other studies in sheep implementing low energy contents in the diet showed sex-related modifications in the offspring. Specifically, it was evidenced a greater influence on male offspring’s reproductive capacity after being exposed to undernutrition during early gestation (up to 95 d post-mating) (Rae et al., 2002) as well as during the periconceptional period (-61 to +30 d relative to mating), accompanied by greater fatness (Jaquiery et al., 2012).

CHAPTER 3

Hypotheses and objectives

3.1. Main hypotheses

The main hypotheses of the present thesis were:

1. Ambient temperatures outside the comfort zone would induce physiological and metabolic changes that could negatively affect production, reproduction, and metabolism in dairy goats.
2. Heat stress during the prenatal life may condition the behavior responses to heat stress or other different kinds of stress during the postnatal life of goat kids.
3. Heat stress during the prenatal life may affect physiological, performance and metabolism of goat kids when exposed to high ambient temperatures during the postnatal life.

3.2. Objectives

The main objective of the current thesis was to understand the effect of extreme temperatures via direct exposure on productive variables and metabolism in dairy goats. Further, the effect of the indirect exposure to in utero heat stress (transgenerational) on growth, metabolism, and behavior of growing goats during the postnatal life were evaluated when they challenged with heat stress and other stimulators.

Therefore, the specific objectives were to evaluate the effects of:

- Evaluate the effects of low ambient temperatures on physiological responses, milk production, and blood metabolite profile in dairy goats. (Chapter 4).
- Studying the impact of heat stress in goats at the beginning of the pregnancy on the gestation performance and the changes in the behavior of the offspring when challenged different environmental stimuli including high ambient temperatures (Chapter 5).
- Determine whether kids of goats exposed to HS during mating and the first 45 days of pregnancy would show different physiological, productive, and metabolic response to HS compared to kids born to dams without HS during pregnancy (Chapter 6).

CHAPTER 4

Physiological, lactational, and blood metabolite profile of dairy goats exposed to low ambient temperatures

4.1. Abstract

Low winter temperatures in some regions combined with increasingly frequent extreme cold waves have a negative impact on animal performance, behavior, and welfare. The objective of this study was to evaluate the physiological, metabolic, and lactational responses of dairy to low ambient temperatures during 3 weeks. Eight Murciano-Granadina dairy goats in mid-lactation were divided into balanced groups and randomly assigned to 2 treatments: thermoneutral (TN; 15 to 20 °C) and low temperature (LT; -3 to 6 °C). The experimental design was a crossover with 2 treatments in 2 periods (21 days each). Goats received a total mixed ration (70% forage and 30% concentrate) and water ad libitum and were machine milked twice daily (8 and 17 h). Feed intake, water consumption, rectal temperature, and respiration rate were recorded daily. Milk samples for composition were collected weekly. Insulin, glucose, non-esterified fatty acids (NEFA), beta-hydroxybutyrate (BHBA), cholesterol, and triglycerides were measured in blood. Body weight (BW) was recorded at the start and end of each period. Compared to TN goats, LT goats had similar feed intake and milk yield, but lower ($P < 0.05$) water consumption (−23%), respiratory rate (−6 breaths/min) and rectal temperature (−0.32 °C). Furthermore, milk of LT goats had greater ($P < 0.05$) contents of fat (+12%), protein (+11%), and lactose (+4%). The LT goats lost −0.45 kg BW, whereas TN goats gained 2.2 kg ($P < 0.05$). Insulin and cholesterol blood levels were not affected by LT. However, values of blood glucose, NEFA, hematocrit and haemoglobin were increased ($P < 0.05$) by LT, whereas BHBA and triglycerides decreased ($P < 0.05$). In conclusion, Murciano-Granadina dairy goats during lactation were sensitive to cold temperatures. The LT goats maintained feed intake and milk production, but produced milk with greater fat and protein contents. Significant changes in metabolism induced by low ambient temperatures, including the increment in NEFA and glucose blood levels, The LT goats mobilized body fat reserves to cover the extra energy needed for heat production under cold conditions.

4.2. Introduction

Low winter temperatures in some regions combined with increasingly frequent extreme cold waves have a negative impact on the production, behavior, and welfare of animals (Kang et al., 2016; Ramón et al., 2016; Salama et al., 2016). The effect is even more severe when animals are maintained outdoor, as they are directly exposed to the inclemency of the weather, which can cause the death under extreme conditions (Bøe and Ehrlenbruch, 2013). Goats are commonly described as rustic and adaptable animals, and they have attributed a wide range of thermal tolerance (Sejian and Srivastava, 2010; Arfuso et al., 2016). Therefore, goats are spread worldwide and raised under different production systems. However, research argues that goats are adversely affected by cold temperatures with different extents of thermal tolerance depending on the breed (McGregor, 2002; Bøe and Ehrlenbruch, 2013).

Some studies have demonstrated that ruminants exposed to cold environments can cope with cold stress by physiological and behavioral changes that allow them to maintain their energy homeostasis, although these responses result in decreased performance and production efficiency (Ekpe and Christopherson, 2000; Verbeek et al., 2012; Kour et al., 2017). In this sense, previous studies focused on cows exposed to cold temperatures reported an increase in feed intake (Curtis, 1981; Young, 1983) and heat production (Graham et al., 1981), a decline in milk yield (Brouček et al., 1991), an immune depression (Kang et al., 2016), and a damage of the peripheral tissue such as frostbit teats (Battini et al., 2014; Yadav et al., 2016).

Compared to other species, especially cows, few studies have been carried out to evaluate the impact of low ambient temperatures on dairy goats. In fact, most of the studies published in goats (Thompson and Thomson, 1972; Wentzel et al., 1979; Faulkner et al., 1980) tested the effects of cold temperatures for short-term (2 to 3 days) and little is known on the long-term effects, especially on metabolism indicators under controlled environmental conditions. In addition, thermoregulatory mechanisms and limits of thermal comfort are not well documented in goats (Bøe et al., 2007; Battini et al., 2014; Yadav et al., 2016).

Our hypothesis was that exposing dairy goats to low ambient temperatures in controlled climatic chamber conditions would allow us to measure precisely the productive and metabolic changes due to cold environment at long-term (i.e. 3 weeks). The objective of the present study was to evaluate the effect of low ambient temperatures on physiological responses, milk production, and blood metabolite profile in Murciano-Granadina dairy goats.

4.3. Materials and methods

4.3.1. Animals, treatments, and management conditions

Animal care conditions, treatment, housing, and management practices followed the procedures stated by the Ethical Committee of Animals and Humans Experimentation of the Universitat Autònoma de Barcelona (UAB) and the codes of recommendations for the welfare of livestock of the Ministry of Agriculture, Food and Environment of Spain.

Eight lactating Murciano-Granadina dairy goats with healthy and symmetrical udders, from the herd of the experimental farm of the UAB were enrolled in the current study. At the start of the experiment, goats were on average (± standard error: 41.8 ± 2.0 kg body weight (BW), 2.13 ± 0.36 L/d milk, and 70 ± 2 days in milk (DIM). Goats were blocked in 2 balanced groups (n = 4/group) according to BW, milk yield and DIM. Goats had 2-wk pre-experimental period to adapt to the experimental conditions.

The experimental design was a crossover with 2 treatments in 2 periods, lasting 21 d, and 4 goats each. Goats were switched to the opposite treatment in the second period. Treatments were: thermoneutral (TN; 15 to 20ºC), and low temperature (LT; -3 to 6ºC with RH = 63% and THI = 44). The change in temperatures for LT was gradual (0.75ºC/h) with minimum and maximum temperatures at 0100 and 1300 h, respectively. The lower critical temperature for goats is considered as 0ºC (Constantinou, 1987). Consequently, our goats remained for 8 h under this presumable limit of cold stress. During the experimental period (mid-January to mid-March), ambient temperatures for TN was controlled by an electric heater provided with a thermostat (3.5 kW; General Electric, Barcelona, Spain). The LT goats were kept in a 4 × 6 × 2.3-m climatic chamber (Euroshield; ETS Lindgren-Euroshield Oy, Eura, Finland) provided with refrigeration system (Model STL200Z012; Rivacold SRL, Vallefoglia, Italy) and programmable relay (Logo; Siemens, Munich, Germany). The environmental temperature and humidity were recorded every 10 min throughout the experiment by data loggers (Opus 10; Lufft, Fellbach, Germany).

Feed was provided individually ad libitum as a total mixed ration consisting of 70% alfalfa hay and 30% concentrate (cracked oat grain 5.0%, cracked corn grain 4.0%, brewing barley 10.0%, soybean hull 45.0%, soybean meal 44%, 5.0%, rapeseed meal 10.0%, corn gluten feed 10.0%, soybean oil 5.0%, cane molasses 2.0%, salt (NaCl) 0.5%, vitamin minerals for goats 1.0%, phosphate bicalcique 2.5%). Clean water and mineral blocks were freely available for each goat (composition: 36.74% Na, 0.32% Ca, 1.09% Mg, 5 g Zn/kg, 1.50 g Mn/kg, 912 mg S/kg, 304 mg Fe/kg, 75 mg I/kg, 50 mg Co/kg, and 25 mg Se/kg; Ovi Bloc; Sal Cupido, Terrassa, Spain). Daily ration was offered twice a day in 2 portions at 0930 and 1530 h. Feed refusals were collected and weighed at 0900 h, and the amount of feed offered was adjusted to allow a 15-20% refusals.

Goats were milked twice daily at 0800 and 1700 h with a portable milking machine (Westfalia Separator Ibérica S.A, Granollers, Spain) set at 40 kPa, 90 pulses/min, and 66% pulsation ratio. Milking routine included cluster attachment without teat cleaning or udder preparation, machine milking, machine stripping, and teat dipping in an iodine solution (P3-io shield; Ecolab Hispano-Portuguesa S. L., Barcelona, Spain).

4.3.2. Sampling, measurements and analyses

Rectal temperature and respiratory rate. Rectal temperature (RT) and respiratory rate (RR) were recorded daily at 0800, 1200 and 1700 h. The RT was measured with a digital veterinary thermometer (model "Accu-vet, ST714AC"; Tecnovet S.L., Barcelona, Spain; range, 32 to 42ºC, and accuracy ± 0.10ºC). The RR was recorded as the number of breaths per minute by counting the flank movements with the help of a stopwatch.

Feed intake and water consumption. Feed intake and water consumption were recorded daily using electronic scale (model Fv-60K; A&D Mercury PTY, Thebarthon, Australia; accuracy ± 20 g). Feed intake and water consumption were determined by the difference between the weight of the amount offered and the amount refused. Trays with sawdust were placed beneath the drinking troughs and weighted daily to take into account water spilled. Feed samples were collected at the beginning of each period and stored at 4ºC for composition analysis. Feed samples were ground through a 1-mm stainless steel screen, and then analyzed for dry matter (DM), acid-detergent fiber (ADF), neutral-detergent fiber (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 crude protein (CP) was calculated as percentage of N × 6.25. The chemical composition and nutritive value of ration ingredients are shown in Table 4.1.

Goats were weighted at the start and end of each experimental period using electronic scale (Tru-Test AG500 Digital Indicator, Auckland, New Zealand; accuracy ± 20 g) to measure the change in BW.

Table 4.1. Chemical composition and the nutritive value (DM basis) of the ration fed to dairy goats.

Item	**Total mixed ration**
Component, %	
Dry matter	81.8
Organic matter	81.7
Crude protein	16.6
Neutral-detergent fiber	33.5
Acid-detergent fiber	23.2
Nutritive value[1]	
UEL,[2] /kg	1.25
UFL,[3] /kg	0.78
NEL, Mcal/kg	1.37
PDI, g/kg	101
PDIA, g/kg	68.6
RPB, g/kg	25.1
Ca_{abs}, g/kg	2.78
P_{abs}, g/kg	5.16

Legend: NEL net energy for lactation; PDI protein digestible in the small intestine; PDIA protein digestible in the small intestine supplied by rumen-undegraded protein; RPB rumen protein balance; UEL units for dairy goats; UFL forage unit for lactation.

[1] Calculated according to the Institut National de la Recherche Agronomique (INRA, 2018).

[2] 1 UEL = 1 kg DM of reference grass.

[3] 1 UFL = 1.76 Mcal of NEL.

Milk yield and milk composition. Milk yield of individual goats was recorded at each milking, whereas milk composition was evaluated weekly. At d 7, 14, and 21 of each period, 50 mL of composited milk samples (from the a.m. and p.m. milkings) were collected and preserved with an antimicrobial tablet (Bronopol, Broad Spectrum Microtabs II; D & F Control Systems Inc., San Ramon, CA) and stored at 4°C until analysis. Refrigerated milk samples were sent to the Laboratori Interprofessional Lleter de Catalunya (Allic, Cabrils, Barcelona, Spain) for the analyses of total solids (TS), fat, protein (N × 6.38), lactose, and somatic cell counts (SCC) using Milkoscan (MilkoScan FT2 – infrared milk analyzer, Foss 260, DK-3400 Hillerød, Denmark) and an automatic cell counter (Fossomatic 5000, Foss Electric, Hillerød, Denmark) previously calibrated for goat milk.

Blood measures. Blood samples were collected at d 3, 7, 14, and 21 of each period at 0800 h before the a.m. feeding and milking from the jugular vein into a 10-mL tube with spray-coated sodium heparin and 10-mL tube with spray-coated K_2-EDTA (BD Diagnostics, Franklin Lakes, NJ). Plasma was obtained by centrifugation for 15 min at 1500 × *g* and 4ºC, and kept at –30°C until the analysis of glucose, insulin, non-esterified fatty acids (NEFA), beta-hydroxybutyrate (BHB), triglycerides, and cholesterol. Glucose was measured by the hexokinase method using an automatic analyzer system (Olympus AU400, Hamburg, Germany). Insulin concentrations were determined using an ELISA kit (Mercodia Ovine Insulin ELISA; Mercodia, AD Bioinstruments, Barcelona, Spain). The NEFA were determined by the colorimetric enzymatic test ACS-ACOD method using a commercial kit (Wako Chemicals, Neuss, Germany). The BHB was determined by a kinetic enzymatic method using a commercial kit (Ranbut, Randox, UK). Triglycerides were analyzed with an enzymatic method (glycerol phosphate oxidase), and cholesterol was also analyzed by an enzymatic method (cholesterol esterase/peroxidase).

At d 7 and 21 of each period additional blood samples (roughly 0.3 mL) were collected using insulin syringes (BD Micro-Fine; BD Medical-Diabetes Care, Franklin Lakes, NJ) at 0800 h before morning feeding and milking, 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 Inc., Princeton, NJ). Then, the cartridge was inserted into an i-STAT hand-held analyzer, and the results of urea, Na, K, Cl, total CO_2 concentration, anion gap (AnGap), hematocrit, hemoglobin, pH, partial pressure of CO_2 (pCO_2), HCO_3^-, and base excess were obtained.

4.3.3. Statistical analyses

Data were analyzed by the PROC MIXED procedure of SAS version 9.1.3 (SAS Institute Inc., Cary, NC) for repeated measurements. Daily data were averaged to weekly values for rectal temperature, respiratory rate, feed intake, water consumption and milk yield. The statistical mixed model contained the fixed effects of the treatment (LT vs. TN), week (1 to 3), and period (1 and 2), the random effect of the animal, the interactions treatment × week and treatment

× period, and the residual error. The model took into account the possible carryover effects of previous LT periods through the treatment × period interaction. For rectal temperature and respiratory rate values measured at 0800, 1200 and 1700 h, a fixed effect of measurement day hour was added to the model. Differences between least squares means were determined with the PDIFF test of SAS. Significance was declared at $P < 0.05$ unless otherwise indicated.

4.4. Results and discussion

4.4.1. Rectal temperature and respiratory rate

As shown in Table 4.2, rectal temperatures increased from 0800 to 1700 h by 0.21ºC and 0.79ºC in TN and LT goats ($P < 0.05$), respectively in accordance with the increment in the ambient temperature (range 15 to 20ºC in TN and -3 to 6ºC in LT). Rectal temperatures at 0800 and 1200 h as well as the daily average values were lower ($P < 0.001$) in LT goats than TN (Table 4.2). However, at 1700 h when the ambient temperature reached its highest level for LT (6ºC), the rectal temperature was similar for both TN and LT goats. Studies on sheep indicated that exposure to cold temperatures (up to 2ºC) results in decreased rectal temperatures (Sano et al., 1999; Li et al., 2000) even immediately after 6 h of exposure to 4.5ºC (Verbeek et al., 2012). However, with greater cold ambient temperatures (i.e. 9ºC), Barnett et al. (2015) did not detect changes in RT of sheep.

Table 4.2. Rectal temperature (RT) and respiratory rate (RR) measured at 0800, 1200 and 1700 h in dairy goats under thermal-neutral (TN) and low temperature (LT) conditions.

Item	Treatment[1]		SED[2]	Effect[3] (*P* <)		
	TN	**LT**		**T**	**P**	**T×P[2]**
RT, ºC						
0800 h	38.40	37.75	0.09	0.001	0.552	0.270
1200 h	38.25	37.97	0.08	0.001	0.549	0.524
1700 h	38.61	38.54	0.07	0.250	0.007	0.360
Average	38.42	38.09	0.07	0.001	0.052	0.308
RR, breaths/min						
0800 h	31	26	0.6	0.001	0.038	0.023
1200 h	32	26	0.6	0.001	0.012	0.080
1700 h	34	27	0.9	0.001	0.141	0.101
Average	32	26	0.6	0.001	0.001	0.041

[1] Values are presented as least-square means. [2] SED standard error of the difference.

[3] Effects of treatment (T), period (P) and their interaction (T x P).

Respiratory rate was lower (*P* < 0.001) in LT goats compared to TN throughout the experimental period (Table 4.2). This agrees with the results of Sano et al. (1999) in sheep exposed to cold environment (2ºC). The decreased respiration rate could be part of acclimation to cold temperatures by which animals decrease evaporative heat loss through the respiratory tract (Sasaki and Takahashi, 1980).

4.4.2. Feed intake, water consumption, and body weight change

The DM intake did not vary (*P* = 0.128) between TN and LT goats (Table 4.3). We expected increased DM intake to meet the increment in energy requirements for heat production. However, it is possible that the feed intake was at its maximum capacity and goats were not able to eat more. In fact, rumen volume has been reported to decrease in cold temperatures (Chase, 2016).

Table 4.3. Productive and physiological parameters (from daily measures) of Murciano-Granadina dairy goats under thermal-neutral (TN) or low temperature (LT) conditions.

Item	Treatment[1]		SED[2]	Effect[3] (*P* <)		
	TN	**LT**		**T**	**P**	**T×P**
ΔBW, kg	2.20	-0.45	0.77	0.031	0.060	0.097
DMI, kg/d	2.71	2.57	0.08	0.128	0.042	0.099
Water consumption, L/d	5.34	4.13	0.25	0.001	0.013	0.401
Water : DMI ratio	1.96	1.59	0.07	0.001	0.061	0.844
Milk yield, kg/d	1.88	1.72	0.09	0.221	0.445	0.583
Energy corrected milk,[4] kg/d	2.12	2.06	0.14	0.695	0.122	0.233
Milk composition, %						
Total solids	13.0	14.1	0.22	0.001	0.164	0.869
Fat	4.47	5.01	0.13	0.001	0.831	0.325
Protein	3.41	3.77	0.13	0.001	0.014	0.094
Lactose	4.53	4.70	0.05	0.001	0.051	0.021
Fat yield, g/d	82.1	82.7	4.3	0.921	0.137	0.380
Protein yield, g/d	62.3	62.4	2.4	0.994	0.045	0.001
Lactose yield g/d	83.5	78.2	4.6	0.113	0.007	0.561
Log SCC	6.22	6.34	0.12	0.169	0.615	0.380

[1] Values are presented as least-square means.

[2] SED standard error of the difference.

[3] Effects of treatment (T), period (P) and their interaction (T x P).

[4] 3.5% fat, 3.2% protein ECM = kg milk * 0.327 + kg fat * 12.95 + kg protein * 7. 2

Similar to our results, Thompson and Thomson (1972) in short-term experiment (2 d) detected no effect of low ambient temperature (0.5 to 1ºC with or without blown air) on feed intake of goats. Additionally, Bøe and Ehrlenbruch (2013) reported that inclement cold weather had no effect on time spent feeding in goats. Sheep exposed to 9ºC have similar DM intake to those animals under 26ºC (Barnett et al., 2015).

Another explanation for the no change in DM intake is that the severity of cold (i.e. -3 to 6 °C) in the current study was not enough to induce a change in feed intake. However, some studies showed that animals exposed to low temperatures increase their feed intake in order to increase metabolic heat production (Young, 1981; Kennedy, 1985; Gaughan and Mader, 2009).

Water consumption as well as water : DM intake ratio were decreased ($P < 0.001$) by 23 and 19%, respectively in LT goats. Similarly, water consumption was reduced by 46 to 62% in goats exposed 0.5 to 1ºC (Thompson and Thomson, 1972). Respiratory rate decreased and consequently water evaporation was declined, which explain the reduced need for water consumption.

Despite similar feed intake, TN goats gained body weight (2.20 kg) at the end of the experimental period, whereas LT goats lost 0.45 kg ($P = 0.031$). This result would indicate that LT goats needed more energy to produce heat, and they mobilized body fat reserves as indicated by the increased blood NEFA (see later).

4.4.3. Milk yield and composition

The effects of cold temperatures on milk yield and composition are shown in Table 4.3. Milk yield and energy-corrected milk were not altered ($P > 0.10$) by low ambient temperature. Similarly, McBride and Christopherson (1984) reported that milk yield in shorn ewes is not affected by low ambient temperatures (0ºC) for 8 wk. In contrast to our results, Faulkner et al. (1980) and Thompson and Thomson (1972) observed a decline in milk yield of dairy goats exposed to cold temperatures. Additionally, Brouček et al. (1991) also reported decreases of 2 kg in milk yield of cows when the temperature was continuously -10ºC for 2 months.

We detected significant treatment × week interaction ($P < 0.05$) due to the fact that milk yield of TN was maintained throughout the experiment, whereas LT goats experienced a tendency of decreased milk yield during the 3rd week (-12%; $P = 0.09$; Figure 4.1). This tendency of decreased milk yield in LT goats could be a consequence of an increment in maintenance requirements by cold environment as goats increase heat production to keep body temperature, which results in less amount of energy available for milk production (especially that feed intake did not increase). Thompson and Thomson (1972) reported that exposure of goats to 0.5 to 1.0ºC with still or blown air increase heat production metabolic rate 18 and 46%, respectively, above that in a thermo-neutral environment. Heat production also increases by up to 55% in shorn ewes kept at 0ºC (McBride and Christopherson, 1984). Furthermore, this decrease in milk yield during week 3 could be due to decreased prolactin secretion. Tucker and Wettemann (1976) reported that heifers exposed to cold temperatures experience reduced blood concentrations of prolactin and growth hormone, and both hormones are important for lactation in goats (Cowie, 1971; Hart, 1973).

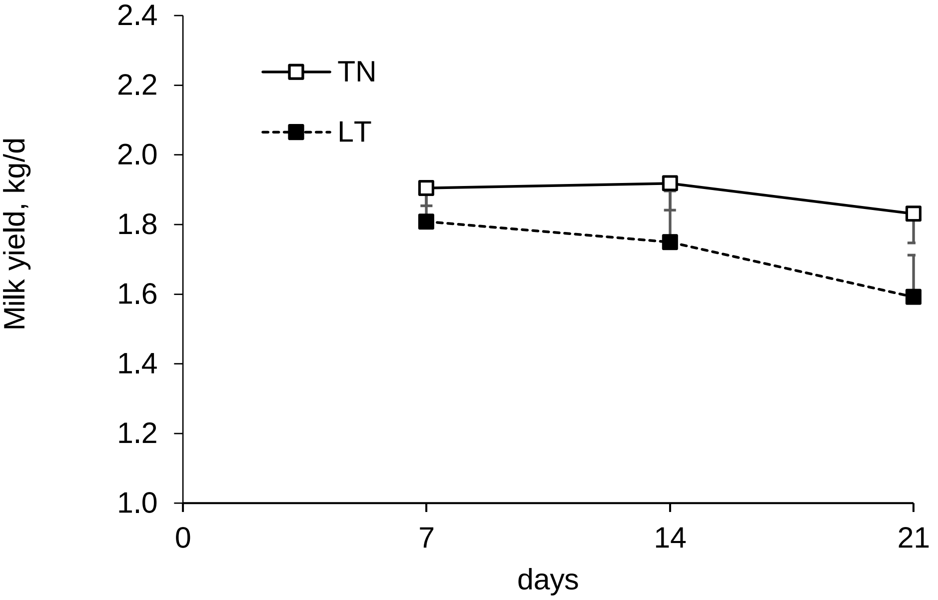


Figure 4.1. Weekly milk yield of dairy goats under thermal-neutral (TN) or low temperature (LT) conditions. Bars indicate standard errors.

The LT goats produced milk with greater (*P* < 0.001) contents of fat, protein and lactose, whereas milk SCC did not vary (Table 4.3). In agreement with our results, McBride and Christopherson (1984) found that shorn ewes exposed to 0ºC produce milk with greater fat, protein and lactose contents. Several studies have demonstrated that milk fat content increases when dairy cows are exposed to low ambient temperatures (reviewed by Johnson, 1976). Mammary uptake of free fatty acids increased dramatically in goats exposed to low ambient temperatures (Thomson et al., 1979). The fatty acids resulting from mobilized lipid tissue were used by the mammary gland for fat secretion, which explains why milk fat content increased in LT goats.

The TN and LT yielded similar amounts of fat and protein (Table 4.3). The fact that LT goats produced similar yields of milk components despite producing numerically 9% less milk (P = 0.221) might indicate that the increment in the percentages of milk components in LT is partially due to a concentration effect (LT goats drank less water and produced more concentrated milk). Although glucose in the circulation was increased in LT goats (see later), there was a numerical reduction in lactose secretion (-6%; P = 0.113), which could be due to the fact that the extraction of glucose from the circulation by the udder falls in the cold environment (Faulkner et al., 1980).

4.4.4. Blood indicators

Values of main blood metabolites in TN and LT goats are shown in Table 4.4 and Figure 4.2. Compared to TN goats, LT goats had greater ($P < 0.01$) values of glucose and NEFA, but lower ($P < 0.01$) values of BHB and triglycerides. Blood levels of insulin and cholesterol did not vary between the TN and LT goats. Similar to our glucose results, sheep exposed to 2 to 4ºC for 5 days have also greater values of blood glucose concentration and turnover, regardless the level of energy intake (Sano et al., 2007). Additionally, exposure to cold environment increases the output of glucose from the liver (Thompson et al., 1978) and consequently glucose in the circulation is increased (Faulkner et al., 1980). This extra glucose in the circulation was not used for milk lactose as milk lactose yield numerically decreased by 6% (P = 0.113), but it seems that this glucose is used by other tissues (e.g. muscles) to increase heat production.

Blood glucose levels are mainly regulated by insulin, glucagon, growth hormone, and insulin sensitivity. Blood insulin in LT goats was 12% less than in TN, but this difference was not significant (P = 0.382). Other hormones that increase in cold temperatures, such as epinephrine (Sano et al., 1996), triiodothyronine (Barnett et al., 2015), thyroxine (Sasaki and Weekes, 1986) and cortisol (Faulkner et al., 1980) enhance glucose production and may synergistically result in increased blood glucose during cold exposure.

Table 4.4. Plasma insulin and metabolites in dairy goats under thermal-neutral (TN) or low temperature (LT) conditions.

Item	Treatment[1]		SED[2]	Effect[3] (P <)		
	TN	**LT**		**T**	**P**	**T×P**
Insulin, mg/L	0.332	0.291	0.058	0.382	0.490	0.405
Glucose, mg/dL	64.1	66.7	1.000	0.005	0.769	0.083
Non esterified fatty acids, mmol/L	0.107	0.215	0.025	0.004	0.059	0.086
ß-hydroxybutyrate, mmol/L	0.528	0.400	0.035	0.001	0.794	0.011
Triglycerides, mg/dL	22.0	18.2	0.855	0.003	0.002	0.107
Cholesterol, mg/dL	86.5	83.7	5.519	0.618	0.454	0.179

[1] Values are presented as least-square means.

[2] SED standard error of the difference.

[3] Effects of treatment (T), period (P) and their interaction (T x P).

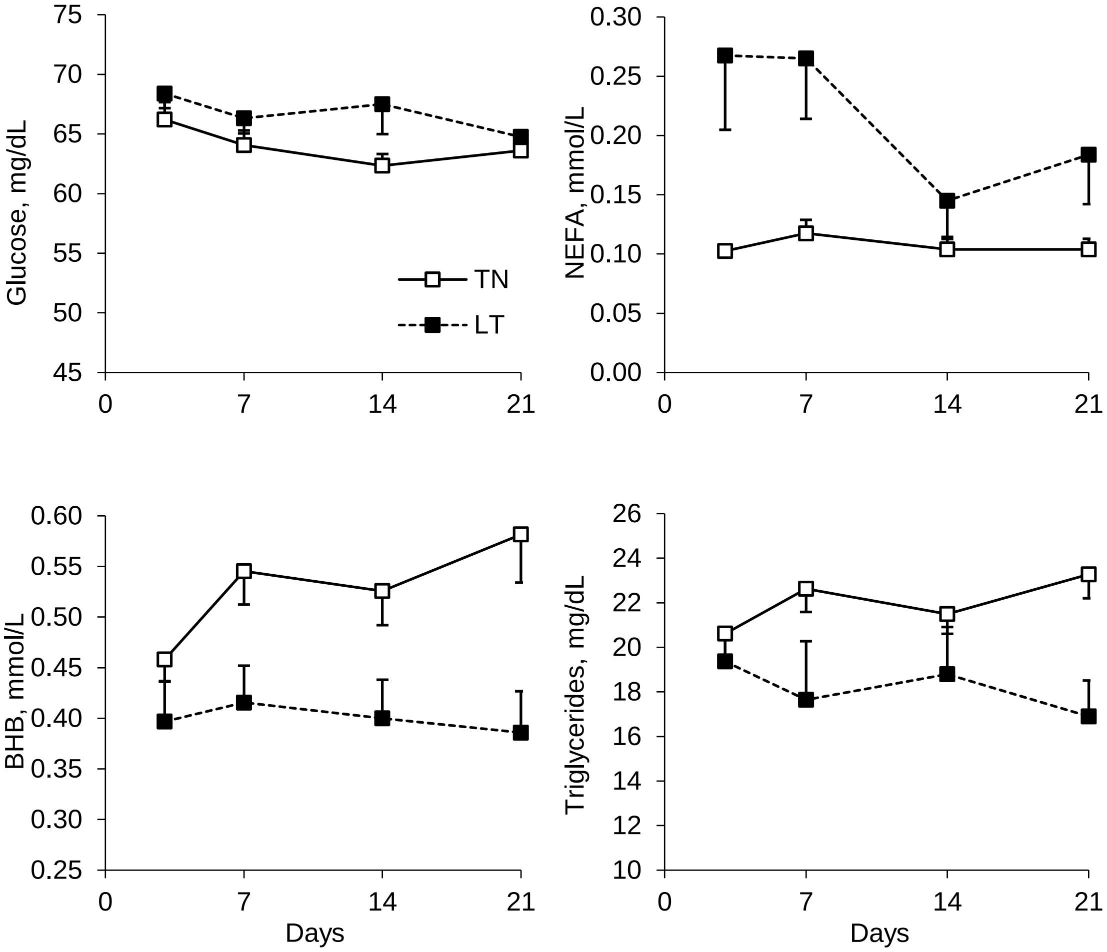


Figure 4.2. Plasma glucose, non-esterified fatty acids (NEFA), beta-hydroxybutyrate (BHB), and triglycerides concentrations in dairy goats under thermal-neutral (TN) or low temperature (LT) conditions. Bars indicate standard errors.

Compared to TN goats, LT dairy goats had similar feed intake, produced similar amounts of milk, but heat production should have been increased as indicated by McBride and Christopherson (1984). It seems that LT goats compensated for the extra energy needed for heat production by mobilizing body fat, which is evidenced by greater ($P < 0.01$) blood NEFA concentrations (Table 4.4). Cortisol levels in blood of goats increase in the cold environment (Faulkner et al., 1980). Additionally, Sasaki and Weekes (1986) indicated that cold exposure increases catecholamine secretion through the enhanced activity of the hypothalamic-pituitary-adrenal system. Glucocorticoids are known to have lipolytic effects, and favor the mobilization of body lipid tissue and increase glucose levels. Similar to our results, heifers kept under 0ºC have greater basal levels of blood glucose and NEFA compared to heifers under 20ºC (Itoh et al., 1997). As body lipid mobilization includes free fatty acids and glycerol, this glycerol could have been used in the liver to produce glucose (Holtenius and Holtenius, 1996), which may also explain greater blood glucose in LT goats.

We speculate that LT goats increased the oxidation of mobilized free fatty acids to CO_2 in the liver and perhaps also in skeletal muscles, not only to produce energy, but also to avoid both excessive fat accumulation in the liver (fatty liver) and the synthesis of hepatic ketone bodies. This assumption is supported by the fact that blood levels of BHB were lower ($P < 0.001$) in LT compared to TN goats (Table 4.4). Synthesis of NEFA in the liver could cause fat accumulation if the amounts of free fatty acids exceed the liver capacity to oxide them to CO_2 or ketone bodies. As a remedy, some triglycerides could be exported as very low-density lipoproteins (triglycerides and cholesterol). However, it seems that this did not occur in LT goats as blood triglycerides were lower ($P < 0.01$) in LT than TN goats, and blood cholesterol did not vary between both groups (Table 4.4).

We cannot rule out the possibility that blood NEFA were partially taken up by the mammary gland for fat synthesis (although milk fat yield did not change) as commonly observed during the periods of negative energy balance in dairy cows (Adewuyi et al., 2005). Consequently, NEFA were not available for ketone body synthesis in the liver, which is an additional explanation of why LT goats had lower BHB levels (Table 4.4).

The NEFA levels were greater during week 1 than weeks 2 and 3 (Figure 4.2). It seems that goats mobilized body lipid tissue to cover the extra needs of energy under LT conditions. However, it is possible these reserves were lost throughout time and goats were not able to mobilize more lipid tissue, which could explain the reduction in milk production during week 3 (Figure 4.1). Losses in body fat in LT goats throughout the experiment may have resulted in less insulation and a greater susceptibility to cold. This is supported by the fact that LT goats lost BW. The quantity of subcutaneous fat and thickness of the skin are the main factors that determine the internal insulation, whereas hair coat depth, wind, precipitation and mud on the hair are the primary factors that determine the external insulation (Chase, 2016). At the end of cold period, we observed that LT goats had thicker hair, possibly as a protection from the low temperatures, but hair data were not recorded. Rate of wool growth increases in sheep exposed to 9ºC compared to 26ºC (Barnett et al., 2015).

Blood values of Na, K, Cl, pH, and total CO_2, bicarbonate, base excess, and anion gap did not vary (P > 0.10) between TN and LT goats (Table 4.5).

Table 4.5. Blood metabolites and acid-base indicators in dairy goats under thermal-neutral (TN) or low temperature (LT) conditions.

Item	Treatment[1]		SED[2]	Effect[3] (P <)		
	TN	**LT**		**T**	**P**	**T×P**
pH	7.42	7.41	0.01	0.100	0.576	0.904
Urea, mg/dL	24.8	23.3	1.1	0.008	0.808	0.344
Na, mmol/L	144.4	143.5	0.4	0.170	0.036	0.841
K, mmol/L	3.79	3.85	0.13	0.660	0.659	0.132
Cl, mmol/L	105	104	0.8	0.292	0.856	0.374
Hematocrit, %	19.8	21.4	0.7	0.001	0.861	0.702
Hemoglobin,[4] g/dL	6.72	7.29	0.25	0.001	0.961	0.714
Total CO_2,[4] mmol/L	28.0	28.4	0.8	0.612	0.516	0.739
HCO_3, mmol/L	26.7	27.3	0.7	0.503	0.440	0.733
pCO_2,[4] mm of Hg	40.7	43.5	1.2	0.001	0.468	0.795
AnGap,[4] mmol/L	16.2	16.1	0.3	0.735	0.053	0.732
Base excess[4]	2.19	2.63	0.79	0.702	0.549	0.702

[1] Values are presented as least-square means.

[2] SED standard error of the difference.

[3] Effects of treatment (T), period (P) and their interaction (T x P).

[4] Calculated values by the i-STAT device software (Abbott Point of Care Inc., Princeton, NJ).

However, lower (P < 0.01) values of urea were detected in LT goats compared to TN animals. This result agrees with Kour et al. (2017) in goats and with Kang et al. (2016) in cattle steers during the winter. However, Shrikhande et al. (2008) did not find differences between summer and winter. The values of partial pressure of CO_2 were greater (P < 0.001) in LT than in TN goats. This is consistent with the lower respiration rate (Table 4.2) and less wash out of CO_2 in LT goats. Hematocrit and hemoglobin were more elevated (*P* < 0.001) in LT than TN goats, which might indicate a slight dehydration derived from the lower water consumption (Table 4.2). The increase in hematocrit in the current study agrees with results of lactating (Thompson and Thompson, 1972) and non-lactating (Appleman and Delouche, 1958) goats exposed to cold temperatures (0 to 1ºC).

It is worth to mention that we detected significant (*P* < 0.05) interactions between treatment and period for milk lactose content and milk protein yield (Table 4.3), and blood BHB (Table 4.4). These significant interactions could be due to the fact that the effects of cold temperatures were relatively greater in the first than in the second period. As mentioned above, LT goats finished the first period with apparently thicker hair coat and lower body lipid reserves, and probably they took some time to replenish body reserves and normalize hair coat.

4.5. Conclusions

Dairy goats were sensitive to low ambient temperatures with some modifications in their performance and metabolism. The LT had similar feed intake, but lost more body weight compared to TN goats. This loss in body weight could be explained by body lipid mobilization as evidenced by greater blood NEFA. These NEFA did not result in greater BHB levels, which means they were used mainly for energy production in the TCA cycle, or alternatively mammary gland took them up for milk fat synthesis. Milk component contents increased in cold ambient, but yields of fat and protein were similar. Blood insulin levels were similar, but LT goats were able to achieve greater blood glucose levels. This extra glucose was not used by the mammary gland as milk lactose yield was numerically lower in LT goats. Alternatively, this extra glucose could have been used in muscles for heat production.

CHAPTER 5

Prenatal heat stress effects on gestation and postnatal behavior in kid goats

5.1. Abstract

Consequences of heat stress during pregnancy can affect the normal development of the offspring. In the present experiment, 30 Murciano-Granadina dairy goats (41.8 ± 5.7 kg) were exposed to 2 thermal environments varying in temperature-humidity index (THI) from 12 days before mating to 45 days of gestation. The environmental conditions were: gestation under thermal-neutral (TN; THI = 71 ± 3); and gestation under heat stress (HS; THI = 85 ± 3) conditions. At 27 ± 4 days old, female kids exposed to *in utero* TN (IUTN; n = 16) or in utero HS (IUHS; n = 10) were subjected to 2 tests: arena test (AT) and novel object test (NOT), the latter was repeated at 3 months of age. Additionally, 8 months after birth, a subset of IUTH and IUHS growing goats (n = 8 each; 16.8 ± 3.4 kg BW) were exposed to 2 environmental conditions in 2 consecutive periods: a basal thermal-neutral period (THI = 72 ± 3) for 7 days, and a heat-stress period (THI = 87 ± 2) for 21 days. In both periods, feeding, resting, posture, and thermally-associated behaviors were recorded. The gestation length was shortened by 3 days in GHS goats. In the AT, IUHS kids showed a lower number of sniffs ($P < 0.01$) compared to IUTN. In the NOT, IUHS kids also tended to show a lower number of sniffs ($P = 0.09$). During heat exposure, IUTN and IUHS growing goats spent more time resting and exhibited more heat-stress related behaviors such as panting and drinking ($P < 0.001$); however, no differences were observed between both groups. In conclusion, heat stress during the first third of pregnancy shortened gestation length and influenced the exploratory behavior of the kids in the early life. However, behavior responses to heat stress during the adulthood were not affected by the *in utero* thermal treatment.

5.2. Introduction

There is evidence that environmental conditions during pregnancy can modify fetal programming through physiological and epigenetic changes (Vitart and Vambesien-Mailliot, 2007; Schroeder et al., 2009), which permanently modify the behavior, health and productivity of the offspring. Several studies have shown that episodes of stress during the prenatal stage have negative effects on the pregnancy itself, by shortening its duration (Seckl, 2001; Schneider et al., 2002), and on the postnatal life of the offspring by reducing birth weight (Schroeder et al., 2009).

Beyond these effects, maternal stress during pregnancy has shown to have profound effects on the development and function of the hypothalamic-pituitary-adrenal (HPA) axis, and the associated circulating ACTH and cortisol concentrations (Weinstock, 2001). Moreover, recent research suggests that these effects remain in further generations (McGowan and Matthews, 2018). In this regard, most studies using rodent or primate models show that gestational stress results in increased aggressiveness and altered social interactions (Marchlewska-Koj et al., 2003; Estanislau and Morato, 2005; Van den Hove et al., 2014) as well as a reduction in the neuromotor capacities, and exploration and learning (Schneider et al., 2002; Vallée et al., 1997).

In the future, as global warming progresses, an increase in temperatures accompanied by increasingly frequent heat waves is expected (Salama et al., 2014). In the case of ruminants, heat stress during pregnancy has attracted special attention, due to the significant impact on food production (i.e. milk) (Dahl et al., 2017). Furthermore, although literature is scarce, thermal stress during pregnancy is demonstrated to be responsible for the abnormal development of the fetus and cause a harmful effect in the early postpartum period and adulthood. For instance, prenatal heat stress can impair the normal postnatal growth of the offspring, compromise the passive immunity, and also alter the behavioral patterns (Laporta et al., 2017; Fabris et al., 2019). Nevertheless, the previous studies evaluated the impact of maternal heat stress during the late gestation in cows, but little is known about the effects of heat stress during early pregnancy on offspring behavior in dairy animals, including cows and goats. There is strong evidence that fetal programming occurs during early gestation in ruminants, and several environmental and nutritional factors during this period can condition performance of offspring permanently. For instance, adequate maternal nutrition in early gestation is critical for the normal development of all fetal organs and tissues (McGeady et al., 2006). Additionally, exposure of cows to limited nutrition during early gestation resulted in decreased skeletal muscle mass and altered glucose metabolism of offspring (Zhu et al., 2007). Therefore, we hypothesized that heat stress (with its related effects such as altered blood flow, changes in hormone levels, reduced feed intake, etc.) during early gestation would alter performance and response of offspring to environmental stimuli.

Besides the genetic component, environment is extremely important in shaping the animal behavior (Breed and Sanchez, 2010). One of the first changes that can be observed in animals that are under stressful conditions is a change in their behavior repertoire. Within behavior, the way animals react to novel situations is also influenced by the environmental conditions in where they live (Yang et al., 220108). Therefore, behavior is a sensitive measure to investigate changes of perception of the environment. The objective of the present study was to investigate the effect of heat stress in goats at the beginning of the pregnancy on the gestation performance of dams and the changes in the behavior of the offspring both at neonatal and adult stages.

5.3. Materials and methods

The animal care conditions, treatment, housing, and management practices followed the procedures stated by the Ethical Committee of Animals and Humans Experimentation of the UAB (4790) and following the EU legislation (Regulation 2010/63/EC).

5.3.1. Treatments and management conditions of dams

Thirty multiparous lactating Murciano-Granadina dairy goats of 41.8 ± 5.7 kg body weight (BW) from the experimental farm of the UAB were used. Goats were housed in 6 pens (5 × 2.5 m^2) of 5 goats each, distributed equally in 2 adjacent rooms, one for each treatment. Goats were distributed by similar BW within each pen. The present experiment was carried out during spring (March to June). After 2 weeks of adaptation to the experimental conditions, goats were distributed in 2 groups exposed to 2 different climatic conditions (n = 15) from day 12 before mating until day 45 of gestation. The climatic conditions were: thermo-neutral (TN), and heat stress conditions (HS). The TN group was maintained between 15 and 20°C , and 49 ± 8% relative humidity (temperature humidity index, THI = 71 ± 3, calculated according to NRC (NRC, 1971), and HS group for 12-h day at 37 ± 0.5°C and 45 ± 5% relative humidity (THI = 85 ± 3) and 12-h night at 30 ± 0.5°C and 47 ± 2% relative humidity (THI = 80 ± 2). The temperature of the TN group was maintained with the help of one 3.5-kW electric heater equipped with a thermostat (General Electric, Barcelona, Spain) when necessary. The room housing HS animals was equipped with four 3.5-kW electric heaters coupled to thermostat (General Electric). Environmental temperature and humidity were recorded every 10 min throughout the experiment by data loggers (Opus 10, Lufft, Fellbach, Germany). Both treatments were maintained from 12 days before mating until 45 days after mating (early gestation).

For mating, goats were divided into 6 groups (5 goats each), resulting in 3 TN and 3 HS groups. Estrus was synchronized in a way that 2 to 3-day intervals were allowed between each mating group. Synchronization was performed using intravaginal sponges (progesterone P4; Sincropart 30 mg, Ceva Animal Health, Barcelona, Spain) for 12 days followed by the administration of equine-chorionic gonadotropin (eCG, 400 IU; Ceva Animal Health) at the time of sponge withdrawal. The 6 goat groups were naturally mated by the same buck at 2 to 3-day intervals. On the day of mating, the buck served the 5 goats twice in the morning and afternoon. For that purpose, each goat was taken outside the chamber to a raceway and introduced to the buck. Consequently, the male had 2 to 3-d rest periods between mating groups.

Feed was provided *ad libitum* as a total mixed ration (70% alfalfa hay and 30% concentrate). Concentrate contained barley 31.5%, corn 41.5%, soybean meal 44.5%, sodium bicarbonate 1%, calcium phosphate 0.4%, calcium carbonate 0.5%, salt 0.7%, and premix 0.4%; as fed basis. Water was freely available at room temperature. Mineral salt blocks (Na 36.7%, Ca 0.32%, Mg 1.09%, Zn 5 g/kg, Mn 1.5g /kg, S 912 mg/kg, Fe 304 mg/kg, I 75 mg/kg, Co 50 mg/kg, and Se 25 mg/kg; Ovi Bloc, Sal Cupido, Terrassa, Spain) were freely available in each pen throughout the experiment.

Goats were milked twice per day using a mobile milking unit set at 42 kPa, 90 pulses/min, and 66% pulsation ratio. Feed intake was recorded daily, calculated by the difference between the weight of the ration offered and the leftover at the end of the day. Rectal temperature (RT) and respiration rate (RR) were recorded daily 3 times per day at 8, 12, and 17 h. RT was recorded with a digital veterinary thermometer (ST714AC Accu-vet, Tecnovet S.L, Barcelona, Spain). RR was calculated as the number of breaths per minute by counting the flank movements with the help of a chronograph and from a distance of 2 m without disturbing the goats.

Pregnancy was confirmed by transrectal ultrasound at days 21 and 45 after mating, and all goats were confirmed to be pregnant. After 45 days of gestation, all goats were gathered in one group and managed under semi-intensive conditions (grazing 6 h/day and feed complemented when indoors). Two weeks before the expected date of parturition, the goats were weighed and moved to kidding pens for permanent surveillance and parturition assistance. Immediately after birth, kids were separated from the goats and fed with their mothers' colostrum and reared together with milk replacer (150 g/L, Elvor, Saint-Brice, France) with an automatic milk provider (Foerster-technik, Engen, Germany). Pregnancy length and litter size of kids were recorded after parturition. The BW of kids was recorded at birth and every week until 4 weeks old with a digital scale (Tru-Test AG500 Digital Indicator, accuracy, Auckland, New Zealand).

5.3.2. Behavioral tests and measurements on female kids

For the behavioral assessment, female kids at 27 ± 4 days old, from IUTN (n = 16) and IUHS (n = 10) groups were individually exposed to an arena test (AT) for 5 consecutive days, and to a novel object test (NOT) at 48 h after the end of the AT. The NOT test was repeated at 3 months of age. The AT and NOT were carried out into an artificial climatic chamber (Eurosheild, ETS Lindgren-Euroshield Oy, Eura, Finland) in order to avoid sounds from outside and variations of temperature. Both AT and NOT were video recorded for subsequent analysis. Furthermore, behavioral response to a heat challenge was evaluated at 8 months of age using 16 female growing goats (8 IUTN and 8 IUHS).

Arena test (AT). The AT was carried out in a 4 × 4 × 2.3 m^3 arena (w × l × h), in which 9 squares of 1.3 × 1.3 m^2 were painted on the ground with chalk. The access to the arena was through a starting cage of 50 × 50 × 60 cm^3 (w × l × h) separated from the arena by a guillotine door (Fig A in 5.1). On the test day, each kid was randomly selected among the 2 treatments, transported from the nursery to the starting cage and freed 30 s later into the arena. The duration of the test was 8 min and time started to run when the kid was completely inside the arena. The following behavioral parameters were measured: number of squares entered, frequency of jumping and sniffing (nose less than 5 cm from the walls or floor) events, number of vocalizations and time spent moving forward.

Novel object test (NOT). For NOT the same procedure was followed as for AT and the same behavioral measurements were registered. In addition, a road hazard cone (0.5 × 0.7 m^2, w × h) was placed on the floor against the wall opposite to the starting cage (Fig B in 5.1), thereby the latency and the frequency of sniffing events addressed to the novel object were registered. The NOT test was repeated one month later.

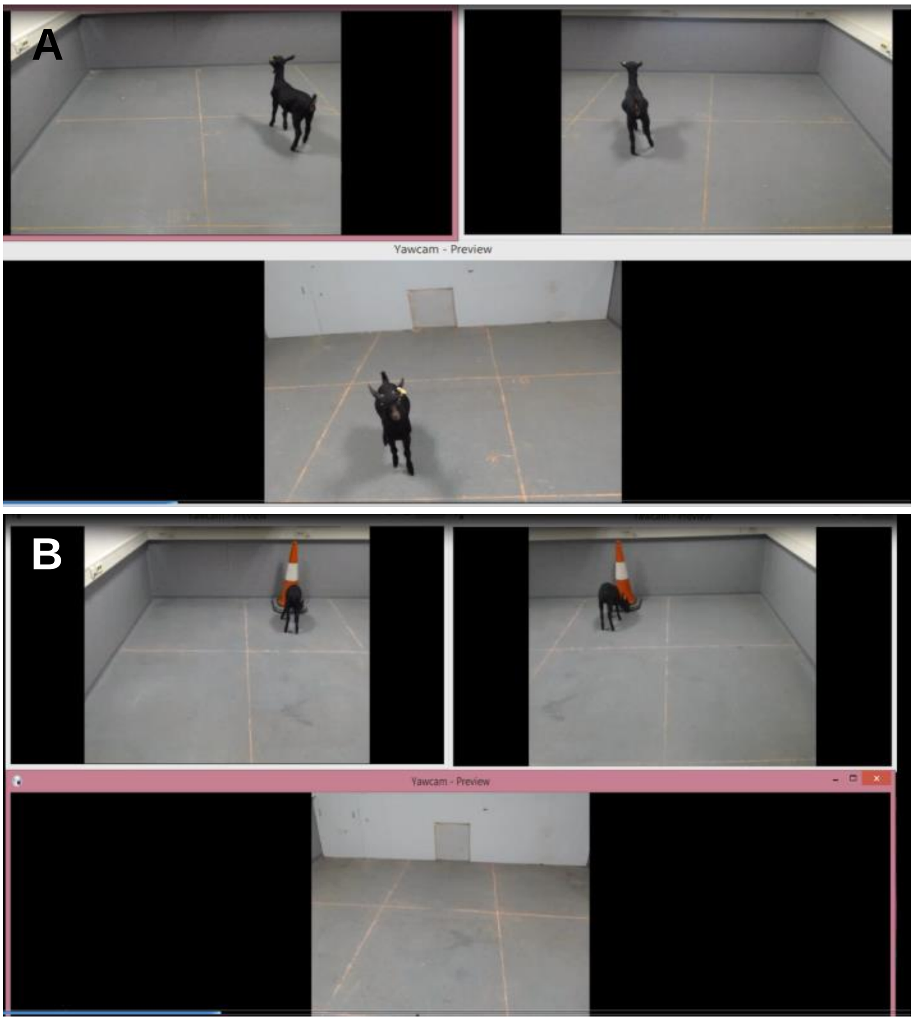


Figure 5.1. Capture of the recording for the arena test (AT) (A). Capture of the recording for the novel object test (NOT) (B).

Heat stress challenge test. To compare the behavioral response of IUTN and IUHS animals to the same stressor (i.e., heat stress) after sexual maturity, a subset of the growing goats was selected at 8 months of age. The IUTN and IUHS goats were balanced by BW and mother parity, and randomly allocated to individual pens (1.08 m^2) with 8 replicates per group in the same room. After one week for adaptation to facilities, 2 different climatic conditions were applied in 2 consecutive periods to both groups. During the first period, basal TN period (1 week), temperature and humidity averaged 24 ± 2.43ºC and 68 ± 9% (THI = 72), respectively. During the second period (3 weeks), goats were exposed to HS conditions, where the average temperature was 37 ± 1.8ºC and humidity was 49 ± 7.0% (THI = 87) during the day and 31 ± 1.4ºC and 53 ± 7.0% (THI = 80), respectively, at night. Room temperature was automatically controlled by 4 electric heaters with a thermostat (3.5 kW; General Electric). Environmental temperature and humidity were continuously recorded every 10 min throughout the experiment by data loggers (Opus 10, Lufft, Fellbach, Germany).

Feed was provided as a total mixed ration consisting of 85% alfalfa hay and 15% concentrate (as feed basis: oat grain 5%, malting barley 10%, canola meal 10%, gluten feed 10%, corn 4.7 %, soy hulls 45%, soybean oil 5%, soybean meal 5%, molasses 2%, bicalcic phosphate 2.5%, salt 0.5%, premix 0.3%) once daily at 9:30 h. Clean water was freely and individually available for each goat.

A single trained observer recorded behavior following a scan-sampling methodology (Martin and Bateson, 2007). Behaviors were recorded between 12 h and 17 h, within the period of heat stress. The behavioral observations were performed daily and the duration of each session was 2 h, whereby each pen was scanned 40 times at 3 min interval.

The behavioral measurements were drawn from the Welfare Assessment Protocol for Goats (Battini et al., 2015). Feeding (feeding + rumination + drinking), other non-feeding active and inactive behaviors (exploration + grooming + other + resting) and physiological behavior associated to thermal stress (open-mouth or close-mouth panting) were recorded as well as posture (standing-walking + standing-immobile + lying-straight + lying-joint). The definition of the recorded behaviors is shown in Table 5.1.

Table 5.1. List of behavioral and postural parameters[1] recorded by scan-sampling during the heat-challenge experiment in the growing goats.

	Description
Feeding behavior	
Feeding	Head in the feeder
Rumination	Chewing rhythmically
Drinking	Mouth at the bucket of water
Non-feeding behaviors	
Exploration	Sniffing the pen, feeder or the bucket of water
Grooming	Grooming itself
Other	None of the previous active behaviors nor resting
Resting	
Thermally-associated behaviors	
Open-mouth panting	Accelerated respiration rate with open mouth
Closed-mouth panting	Accelerated respiration rate with close mouth
Postures	
Standing-walking	Standing up displaying a no resting nor other behavior
Standing-immobile	Standing up displaying other active behavior
Lying-joint	Resting lying with legs drawn into the body
Lying-straight	Resting lying with legs held away from the body
Neck extended	The neck is extended on the floor

[1] These parameters are drawn from the Welfare Assessment Protocol for Goats (Battini et al., 2015).

5.3.4. Statistical analyses

The duration of pregnancy and birth weight were analyzed with the GLM procedure of SAS (version 9.4; SAS Institute Inc., Cary, NC). The feed intake, RT and RR measurements on goats (dams) were analyzed as repeated measures using a linear mixed model (PROC MIXED procedure of SAS). Behavioral data from NOT and scan sampling during the heat exposure test, as counts and week average percentages, respectively, were analyzed as repeated measures using a generalized linear mixed occasional behaviors, using a generalized linear model (PROC GENMOD), all adjusted under a Poisson or a Negative Binomial distribution, according to the fitness of the model. Also, litter size was analyzed using the PROC GENMOD procedure. The models included treatment (HS vs TN for dams and IUHS vs IUTN for kids) as fixed effect, and in the case of repeated measures, day or week was also included as a fixed effect as well as the interaction of treatment × day or treatment × week, while animal was considered as a random effect. Litter size was used as a covariable for the analysis of the duration of pregnancy. For litter weight, the effects treatment, sex, litter size, and treatment × sex interaction were considered in the model. Differences between least squares means were determined with the PDIFF test of SAS. Significance was declared at $P < 0.05$ and trend at $P < 0.10$ unless otherwise indicated.

5.4. Results

5.4.1. Effects of heat stress during the pregnancy and early postpartum

Regarding the physiology measurements of goats during the experimental period, HS goats showed a greater ($P < 0.01$) RT compared to TN goats (average 38.7ºC for TN and 39.3ºC for HS; SED = 0.07) and greater ($P < 0.01$) RR (average 33 breaths/min for TN and 108 breaths/min for HS; SED = 3.06), Feed intake was lower ($P < 0.01$) in HS compared to TN goats (2.52 kg/day for TN and 2.12 kg/day for HS; SED = 0.55).

The results of the different variables evaluated at parturition and early postpartum period are shown in Table 5.2. Although goats in the present study were mated under heat exposure, all animals were effectively fertilized as indicated by the transrectal ultrasonography at days 21 and 45 of pregnancy. However, 4 goats (2 TN and 2 HS) did not deliver kids. These 4 goats suffered fetus losses after the pregnancy diagnosis has been done.

Table 5.2. Gestation length in dams and performance of kids at birth and early postpartum period.

Item	Treatment[1]		SED[2]	Effect (P <)	
	TN **n = 15**	**HS** **n = 15**		**Treatment**	**Litter size[3]**
Litter size	2.31	2.23	0.31	0.806	-
Litter weight, kg	5.40	4.71	0.71	0.061	0.001
Duration of pregnancy, d	146	143	0.9	0.006	0.915
Birth-weight of kids[4], kg	2.34	2.18	0.10	0.122	-
Weight of 35-d-old kids[5], kg	7.88	7.64	0.54	0.520	-

[1] TN, dams exposed to thermal-neutral conditions during the first 45 days of gestation (n = 15); HS, dams exposed to heat-stress during the first 45 days of gestation (n = 15).

[2] Standard error of the difference.

[3] Litter size used as a covariable.

[4] n = 30 kids for TN, and n = 29 for HS.

[5] n = 26 kids for TN, and n = 23 for HS.

The gestation length was on average shortened by 3 days in HS goats compared to TN (*P* = 0.006). Thirty and 29 kids were born to TN and HS goats, respectively. The litter weight of HS group tended (*P* = 0.061) to be lower for HS compared to TN goats despite the fact that more male kids were born to HS (n = 13) than TN (n = 7) goats, and males weighed more than females (2.50 ± 0.10 vs. 2.37 ± 0.07 kg for males and females, respectively; *P* < 0.05). Litter weight was influenced by the litter size (*P* < 0.001), as a greater litter size was associated to smaller kids. However, kids' body weight at 35 days of age was not affected by the treatment (*P* > 0.10).).

5.4.2. Behavioral responses to arena test

The results of the behavioral responses to the AT test are summarized in Table 5.3. A significant day effect was observed for all behavior variables, as the number of vocalizations (*P* < 0.001) and time spent moving forward (*P* = 0.001) decreased, whereas the number of jumping (*P* < 0.001) and sniffing events (*P* = 0.009) increased from day 1 to day 5, reflecting familiarization of kids with the arena test facilities. Further, the number of squares that kids walked through showed to be higher from day 1 to day 2 afterwards being diminished towards day 5 (*P* ≤ 0.001), which is consistent with the reduction in the time spent moving forward.

Table 5.3. Behavioral responses in arena test (AT) of female kids during 5 consecutive days.

Item	Treatment[1]			Effect (*P* <)		
	IUTN **n = 16**	**IUHS** **n = 10**	**SED[2]**	**Trt[3]**	**Day**	**Trt×Day[1]**
No. of squares entered	43.4	31.5	4.87	0.115	0.009	0.704
No. of jumps	1.54	1.15	0.48	0.586	0.001	0.546
No. of sniffs of the arena	33.5	26.7	1.62	0.007	0.001	0.335
No. of vocalizations	171	150	11.7	0.200	0.001	0.001
Time spent moving forward, s	54.8	44.9	7.06	0.282	0.001	0.123

[1] IUTN, kids born to dams exposed to thermal-neutral conditions during the first 45 days of gestation; IUHS, kids born to dams exposed to heat stress conditions during the first 45 days of gestation.

[2] Standard error of the difference.

[3] Trt, treatment effect (IUHS vs IUTN).

Regarding the effect of treatment, IUHS kids showed a lower number of sniffing events compared to GTN kids (*P* = 0.009). Additionally, the significant interaction between treatment and day for vocalizations (P < 0.001) was due to the fact that the number of vocalizations in the IUHS kids was lower during the 2 first days (*P* ≤ 0.05) and recovered thereafter. The rest of behavioral parameters assessed were not influenced by the in utero thermal treatment (*P* > 0.10).

5.4.3. Behavioral responses to novel object test

The novel object test (NOT) was performed at 1 and 3 months of age and results are shown in Table 5.4. At 1 month of age, IUHS kids showed trended to decrease the number of sniffing events compared to IUTN kids (*P* = 0.093), indicating a weaker motivation for exploration of novel objects in kids whose mothers suffered from heat stress during gestation. No treatment differences were detected for the other behavioral variables. At 3 months of age, no treatment effects were found on any of the variables assessed in the NOT.

Table 5.4. Behavioral responses in novel arena test (NOT) of female kids at 1 and 3 months of age. Values are presented as means ± standard deviation.

Item[1]	Treatment[1]		Effect (*P* -value)
	IUTN **n = 16**	**IUHS** **n = 10**	**Treatment**
1 month of age			
No. of squares entered	47.5 ± 1.08	38.9 ± 1.10	0.127
No. of jumps	4.81 ± 1.70	2.30 ± 1.98	0.413
No. of sniffs of the arena	36.1 ± 1.06	30.3 ± 1.08	0.093
No. of vocalizations	156 ± 1.1	162 ± 1.1	0.670
Time spent moving forward, s	48.8 ± 3.87	41.0 ± 4.90	0.220
No. of sniffs of the object	14.8 ± 1.14	10.5 ± 1.19	0.136
Latency before 1st sniff of the objects, s	53.9 ± 53.8	77.4 ± 24.8	0.562
3 months of age			
No. of squares entered	41.3 ± 1.10	41.3 ± 1.13	0.998
No. of jumps	0.31 ± 1.56	0.50 ± 1.56	0.461
No. of sniffs of the arena	30.1 ± 1.06	33.5 ± 1.08	0.286
No. of vocalizations	168 ± 1.0	157 ± 1.0	0.670
Time spent moving forward, s	59.0 ± 6.46	53.0 ± 8.17	0.609
No. of sniffs of the object	5.25 ± 1.15	4.40 ± 1.22	0.136
Latency before 1st sniff of the objects, s	43.2 ± 7.47	40.9 ± 9.96	0.855

1 IUTN, kids born to dams exposed to thermal-neutral conditions during the first 45 days of gestation; IUHS, kids born to dams exposed to heat stress conditions during the first 45 days of gestation.

5.4.4. Behavioral responses to the heat challenge

The behavior variables measured at 8 months of age during the heat stress challenge are summarized in Table 5.5. No differences were observed between IUTN and IUHS goats for any of the variables measured (*P* > 0.10). Only lying-straight showed a treatment per time interaction trend (*P* = 0.099), however, no further differences were found between IUTN and IUHS animals neither during the basal TN period (1 week) nor during the heat-stress period (3 weeks).

Table 5.5. Behavioral and postural average expression of growing goats over the basal thermal neutral period and the heat stress challenge period.

Item	Treatment[1]		SED[2]	Effect (*P* <)		
	IUTN n = 8	**IUHS n = 8**		**Trt[3]**	**Week[4]**	**Trt×Week**
Feeding behavior, %						
Feeding	24.0	25.2	2.14	0.702	0.001	0.533
Rumination	14.1	16.9	1.5	0.179	0.001	0.345
Drinking	2.02	1.58	0.40	0.042	0.001	0.857
Non-feeding behaviors, %						
Exploration	4.46	4.88	0.81	0.709	0.001	0.231
Grooming	3.81	3.77	0.52	0.957	0.001	0.613
Other	3.07	2.77	0.43	0.645	0.003	0.390
Resting	41.2	37.9	2.35	0.312	0.001	0.361
Thermally-associated behavior, %						
Open-mouth panting	0.99	1.18	0.63	0.786	0.001	0.989
Close-mouth panting	41.6	37.0	3.90	0.448	0.001	0.502
Postures, %						
Standing-walking	1.38	1.55	1.55	0.644	0.001	0.985
Standing-immobile	33.6	35.3	2.64	0.643	0.001	0.718
Lying-joint	54.5	50.9	3.82	0.505	0.001	0.703
Lying-straight	4.95	5.48	2.17	0.859	0.001	0.099
Neck extended	0.42	0.23	0.23	0.736	0.006	0.249

[1] IUTN, kids born to dams exposed to thermal-neutral conditions during the first 45 days of gestation; IUHS, kids born to dams exposed to heat stress conditions during the first 45 days of gestation.

[2] Standard error of the difference.

[3] Trt, treatment effect (IUHS vs IUTN).

[4] Basal period corresponded to the first week at thermal-neutral conditions and heat stress (HS) period corresponded to the following three weeks.

All parameters were affected by the heat-stress challenge independent of the in utero thermal treatment as shown in Fig 5.2. Feeding, exploration and grooming behaviors were reduced immediately after the heat challenge (week 2) and remained low compared to the basal TN period (*P* < 0.001) in both, IUTN and IUHS goats. Rumination was also reduced during the heat challenge, but it started to recover towards the end of the experiment although never returned to the basal thermal-neutral values (week 4; *P* < 0.001). Drinking behavior also increased dramatically during the first week of exposure to heat (*P* < 0.001), but returned to initial values at the end of the experiment. Resting also increased progressively throughout the exposure to heat stress although did not reach basal values by the end of the experiment.

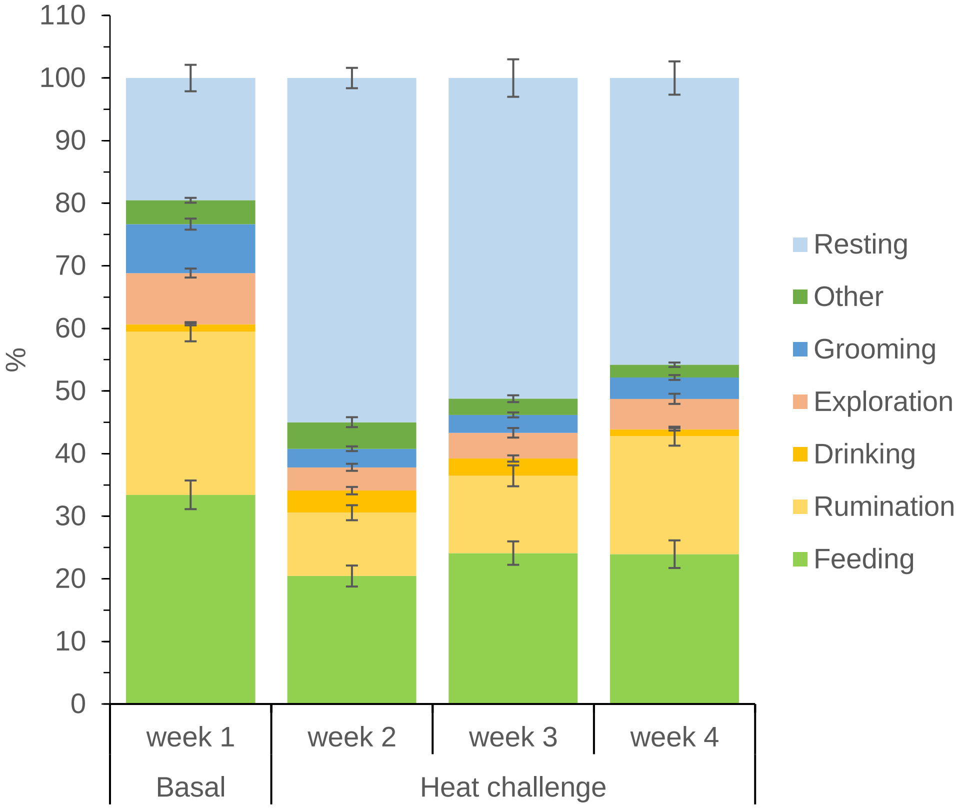


Fig 5.2. Activity behavior average expression (%) of growing goats over the basal thermal-neutral period (week 1) and during the heat-challenge period for 3 weeks (weeks 2 to 4).). Bars indicate standard error.

Postural behaviors averages are presented in Fig 5.3. Animals were lying more frequently during the heat challenge (*P* < 0.01), predominantly with legs joint, which resulted in less (*P* < 0.001) standing (immobile or walking) behaviors.

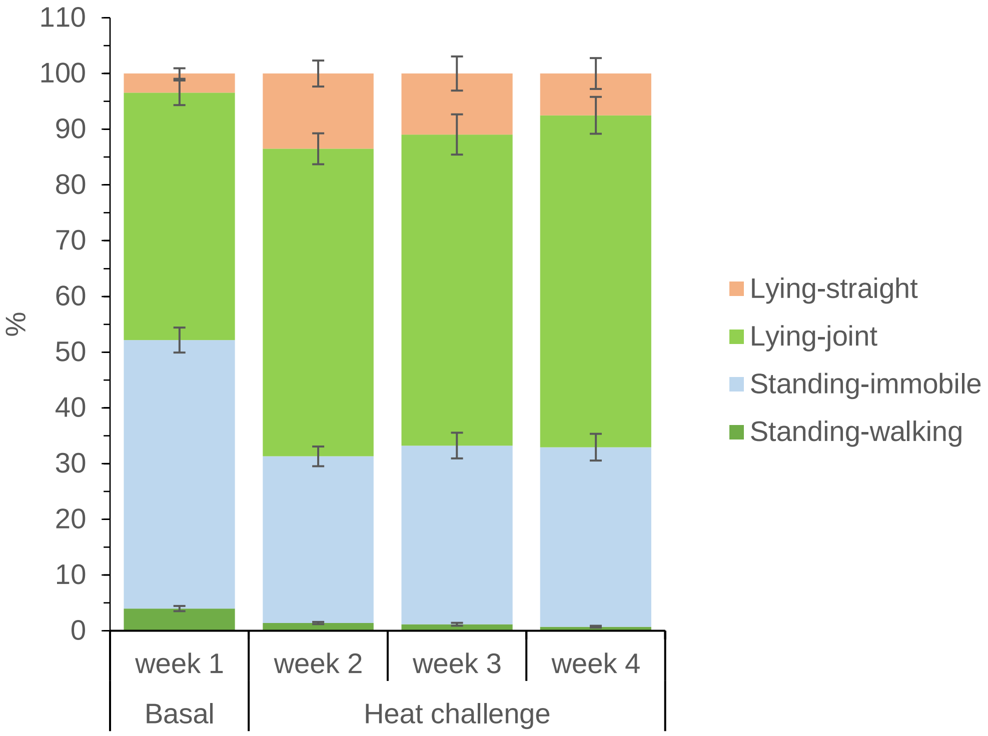


Fig 5.3. Posture average expression (%) of growing goats over the basal thermal-neutral period (week 1) and during the heat-challenge period for 3 weeks (weeks 2 to 4). Bars indicate standard error.

As shown in Fig 5.4, the neck was extended more ($P = 0.018$) frequently during the heat challenge period compared to the basal TN week. Furthermore, goats experienced greater ($P < 0.001$) close-mouth panting after being exposed to heat and reduced ($P < 0.05$) this behavior progressively towards the end of the heat stress period. Open-mouth panting was highest at week 1 of HS and disappeared by week 3 of heat stress.

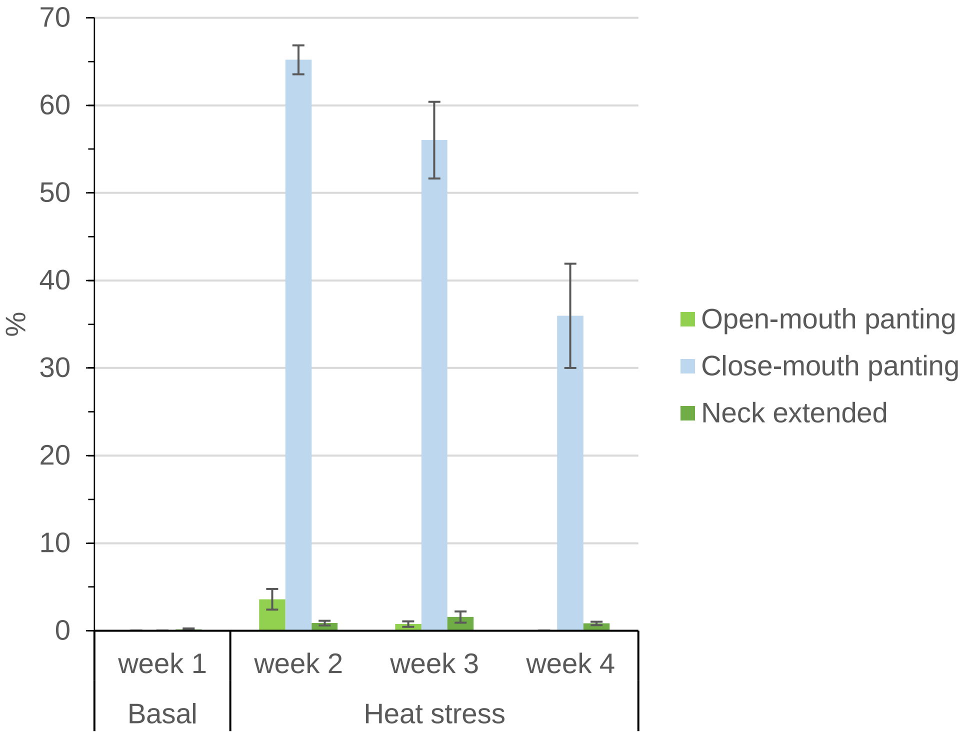


Fig 5.4. Thermally-associated behavior average expression (%) of growing goats over the basal thermal-neutral period (week 1) and during the heat-challenge period for 3 weeks (weeks 2 to 4). Bars indicate standard error.

5.5. Discussion

In the present study the effect of prenatal stress by exposing dairy goats to heat during mating and early pregnancy was evaluated. We investigated whether gestational exposure to heat stress would have an impact on the behavior of the offspring later in life. Our HS pregnant goats experienced greater RT (+0.68ºC) and RR (+76 breaths/min), but lower feed intake by 15% compared to TN goats. These findings indicate that HS treatment effectively triggered a significant heat stress response during pregnancy, which agrees with previous results obtained in goats exposed to HS conditions (Hamzaoui et al., 2013; Castro-Costa et al, 2015; Shilia et al., 2016).

We hypothesized that heat stress during early gestation would influence both gestation and the development of the offspring postnatally. In this regard, the most relevant outcomes were the shortening of the gestation duration of HS goats by 3 days and the tendency of reduced of litter weight of IUHS kids compared to IUTN kids. Although in our study the association between the gestation length and the birth weight could not be confirmed, there is sufficient body of research that has confirmed this link in the past in sheep (Forbes, 1967) and in cows [Laporta et al., 2017; Monteiro et al., 2016). These authors suggested that shorter time of gestation could lead to a reduction in the contributions of nutrients from the mother to the fetus. The last 2 months of the pregnancy period is when the greatest growth of the fetus occurs in cattle (60% of the weight at birth) (Tao and Dahl, 2013), which could partially explain the lower litter weight of IUHS kids. In fact, both the shortening of the pregnancy and thus, the derived prematurity of the animals, and the thermal effect could be cofounded.

On the other hand, other authors (Tao et al., 2014) reported no effect of heat stress during the last 2 months of gestation on pregnancy length in cows, but birth weight of calves born from those heat-stressed cows is reduced. This finding suggests that the reduction in the duration of pregnancy itself is not the sole factor responsible for the decreased birth weight, and there may be other biological changes occurring during the in utero heat stress that affect birth weight. Heat stress during pregnancy is actually associated with poor placental development and lower blood flow, which may result in less nutrient flow to the fetus (Tao and Dahl, 2013; Yates et al., 2011). Additionally, Zhu et al. (2016) reported that nutrient restriction of beef cows during the first third of gestation period results in reduced placental development and fetal weights. Hence, a reduction of nutrient supply during the first third of gestation (less feed intake by HS goats) could result in impaired placental function, which negatively affects growth during gestation and contributes to lower birth weight. Despite the observed differences in litter weight in the current study, body differences between IUHS and IUTN kids at 35 days of life were negligible, which indicates that IUHS kids were able to recover the loss of fetal body growth after birth.

In the present study we exposed IUTN and IUHS female kids to AT and NOT tests in order to assess their behavioral reactivity to a new environment and an unfamiliar object, respectively. The results indicated mild changes in the behavioral response of kids previously exposed to in utero heat stress. During the AT, IUHS kids showed a reduction in the number of sniffing events in the arena. When kids were exposed to NOT at 1 month of age, a reduction in exploratory behavior (i.e. sniffing events) was also confirmed, but these differences disappeared when kids were exposed again to NOT at 3 month of life. These results contrast with those obtained by Roussel et al. (20015) who found that kids born to goats under transport stress explore the new environment (i.e. sniffing) more often than control kids. Some behavioral indicators such as immobilization, a reduction in explorative behavior, and reactivity towards humans have been related to fear (Romeyer and Bouissou, 1992; Ruiz-Miranda and Callard, 1992; Coulon et al., 2014). At the hormonal level, these changes have been associated to alterations in the hypothalamic-pituitary-adrenal (HPA) axis (Schneider et al., 2002) caused by an elevation of cortisol in the maternal circulating blood during the fetal development (Roussel et al., 2005; Coulon et al., 2014). We did not measure blood cortisol levels in our pregnant goats or kids, but Hamzaoui et al. (2013) reported that fecal corticosterone was not affected by chronic HS (i.e. 5 weeks of exposure to HS). It is also worth to mention that most of the development of the neural system takes place during the latter phases of gestation, and in our study, goats were not exposed to heat stress during late gestation. This could be a reason why the differences found in our animals were not similar to what reported in previous reports.

In a longer-term scenario, the effects of prenatal heat stress on kids were followed up in growing goats at 8 months of life. For that purpose, the behavior was assessed by scan-sampling before and after a heat challenge in order to elucidate whether in utero heat stress would have any effect on the response to HS in the postnatal life. Both IUTN and IUHS behaved similarly under TN and HS conditions. However, shifting from TN to HS resulted in significant behavioral changes to cope with the high ambient temperatures regardless the in utero thermal treatment. Resting and drinking increased dramatically during the first week of heat exposure. Lying and drinking are considered as ideal biological markers for assessing the severity of the heat stress response (Shilia et al., 2016). Similarly, exploratory, grooming, and feeding behaviors declined throughout the entire period of heat exposure. Furthermore, rumination, an essential component of the ruminant behavior that is also used as an indicator of stress and anxiety (Herskins et al., 2004; Bristow and Holmes, 2007), was reduced. These activities were also accompanied by changes in the posture of animals, spending longer time lying during heat exposure, and consequently shorter time was spent standing. Lying and inactivity are common behaviors observed during the exposure to high ambient temperatures as a strategy to reduce heat load. In addition, feeding behavior was decreased as a mechanism to reduce heat production (Kaliber et al., 2016; Kovács et al., 2018). Based on the behavioral changes observed in the current study, growing kid goats triggered an acute stress response during the first week of exposure to heat stress. However, the fact that lying and drinking were gradually decreased afterwards suggests that animals were able to progressively adapt to the rise of temperature.

In accordance with the results obtained in the AT and NOT, in which behavioral differences between IUTN and IUHS goats disappeared with age, most of the behavioral responses to the heat challenge at 8 months of age were also not affected by the in utero thermal treatment (no significant interaction between treatment and week). Only a tendency was observed for lying with straight legs (P = 0.099). Similarly, Akbarinejad et al. (Akbarinejad et al., 2017) could not demonstrate changes in the adaptation capacity after submission to heat stress occurring at first, second or last third of gestation in cows. Thence, although it seemed that kids were affected at birth and early age, results observed later in life would suggest that HS during early gestation would not affect the offspring behavior at long term. From the behavioral point of view, heat stress during the first third of gestation may not induce changes in the adaptive capacity of the offspring when exposed to heat challenge during the postnatal life.

5.6. Conclusions

Heat stress during the period of mating until the first 45 days of gestation in dairy goats shortened the duration of pregnancy and tended to reduce the litter weight of kids. The behavioral response of kid goats to a novel environment and objects was altered by the *in utero* heat stress. The exposure of the fetus to a specific stress type (i.e., heat stress) can modify its ability to respond to other types of stress (e.g., environmental stress) in the early postnatal life. Nonetheless, under the conditions of this study (gestation stage at which dams were heat-stressed and heat intensity) such an impact disappeared towards the adult life of the animals with no differences in adaptability to heat stress.

CHAPTER 6

Effect of heat stress on female-kids born to dams exposed to heat stress during the first third of gestation

6.1. Abstract

Little is known on whether heat stress during the fetal period would alter the thermoregulation, performance and metabolism after birth in goats. Female-goats at 8 months of age that suffered in utero heat stress [IUHS; n = 8; temperature-humidity index (THI) = 85 ± 3] for the first 45 days of gestation or exposed to in utero thermal-neutral conditions (IUTN; n = 8; THI = 71 ± 3) were used. At 8 months of age, both IUTN and IUHS goats (16.8 ± 3.4 kg BW on average) were exposed consecutively to 2 environmental conditions: a basal thermal-neutral period (TN; THI = 72 ± 3) for 7 days, and a heat stress period (HS; THI = 87 ± 2) for 21 days. During both periods, rectal temperature (RT), respiratory rate (RR), feed intake, water consumption, growth rate, and blood insulin and metabolites levels were recorded. Additionally, glucose tolerance test (GTT), insulin tolerance test (ITT) and epinephrine challenge were performed during TN and HS periods on 4 IUTH and 4 IUHS goats. Regardless the in utero thermal stress, exposure of goats to heat stress resulted in increased RT, RR and water consumption, but decreased feed intake and growth rate. However, IUHS goats needed 10% less RR to keep the same RT as IUTN animals during the 3-wek HS period. Furthermore, IUHS in hot conditions secreted more insulin in response to glucose infusion, and this insulin stayed for longer time in the circulation compared to IUTN animals. In conclusion, the lower overall RR with similar RT might indicate that IUHS goats are able to loss heat by alternative ways rather than panting. In addition, greater secretion of insulin in response to glucose administration during HS period may suggest that IUHS kids are more prone to have fatter body in the postnatal life if raised in hot conditions.

6.2. Introduction

Evidences gathered over the last few years show a remarkable climate change as well as an increase in the frequency and intensity of heat waves (Hamzaoui et al., 2013; Salama et al., 2014). Among the multiple environmental factors that limit the productivity of livestock animals, heat stress (HS) is the most significant due to its impact on the performance, health, and welfare of animals (Ahmed et al., 2017; Al-Dawood, 2017). In this context, dairy animals under extreme hot conditions have shown severe impairment of production yields and body physiology. On one hand, heat stress reduces feed intake (West, 2003), and consequently growth (Pragna et al., 2018) and milk production (Hamzaoui et al., 2013). In addition, high temperatures alter the thermoregulatory system (Dahl et al., 2017) while force the animals to redistribute the body resources with changes in the energy metabolism (Wang et al., 2016). Furthermore, this extreme environmental condition may affect the passive immunity as well, as observed in calves (Laporta et al., 2017).

To mitigate the negative effects caused by HS, several strategies have been studied. Among them, fetal programming is a recent hot area of research based on the assumption that organogenesis and tissue growth might be influenced by the intrauterine environment (Barker et al., 2002). Therefore, a critical event during pregnancy such as heat stress might alter the normal development of the animals during the fetal period being extended after birth (Dahl et al., 2017). In this sense, fetal programming has been purposed as an approach to improve the thermal tolerance of the animals that may persist after birth and during the adult life, and may allow them to cope with the upcoming high temperatures. For instance, Dahl et al. (2017) observed that calves suffering heat stress in utero during the late gestation exhibited differential methylation patterns in multiple tissues. Furthermore, cows born to dams exposed to HS during the late gestation improved their heat tolerance by reducing rectal temperature and sweating rate after a heat challenge (Ahmed et al., 2017). On the contrary, some studies have shown that HS in utero can exert negative carry-over effects in the fetus that also persist in the adult life. Tao and Dahl (2013) observed a reduction in birth weight of bulls and heifers from mothers that were exposed to HS during the late gestation. Additionally, Monteiro et al. (2016b) reported lower growth rates of calves born to heat-stressed cows during late pregnancy.

To our knowledge, most of the published studies evaluated the effects of in utero HS during the last 2 months of pregnancy in bovine, but scarce information is available regarding the influence of HS during the early gestation on the offspring, especially in goats. Early embryogenesis is a critical period for establishment of epigenotypes, and an imbalance in the key nutrients needed for the methylation process alters the epigenetic patterns (Chmurzynska, 2010). In fact, nutritional restriction during the first few weeks of gestation in sheep resulted in long-term effect on performance, physiology and health of the offspring (Cleal et al., 2007). We hypothesized that in utero HS during the first third of gestation would impact fetal programming, not only by the reduced feed intake (which is a typical response to HS), but also by a direct effect of high temperature. Thus, we expected that heat stress in utero would alter the thermoregulation and physiological responses to heat stress later in life. Therefore, the objective of this study was to determine whether kids of goats exposed to HS during mating and the first 45 days of pregnancy would show different physiological and metabolic response to HS compared to kids born to dams without HS during pregnancy.

6.3. Materials and methods

The animal care conditions, treatment, housing, and management practices followed the procedures stated by the Ethical Committee of Animals and Humans Experimentation of the UAB (CEAAH 4790) and following the EU legislation (Regulation 2010/63/EC).

6.3.1. Animals, treatments, and management conditions

Animals used in the current experiment were a subset of those IUTN and IUHS kids used in experiment 2. Briefly, Murciano-Granadina goats exposed to two climatic conditions: thermo-neutral (TN; 15–20°C; n = 15); or heat stress (HS; 12-h day at 37.0 ± 0.5°C and 12-h night at 30 ± 0.5°C; n = 15) during the mating period and first 45 d of the pregnancy. This resulted in goat-kids that were exposed to in utero TN (IUTN) and in utero HS (IUHS).

At 8 months old, 8 IUTN female goats (16.9 ± 0.66 kg BW) and 8 IUHS female goats (16.6 ± 1.01 kg BW) were used. Both groups had similar mother parity and litter size. Goats were adapted for the experimental conditions for 1 week. Afterwards, both goat groups were exposed to 2 different environmental treatments in 2 consecutive periods: TN period (P1) for 7 days as a basal period at 20°C, and HS period (P2) 21 d at 37°C for 12 h and 30ºC for 12 h.

During the experimental period, all goats were housed indoor in the same room 4×6×2.3 m^3 (individual pens; 1.08 m^2), equipped with 4 electric heaters coupled to thermostat (3.5 kW; General Electric, Barcelona, Spain). Environmental temperature and humidity were recorded every 10 min throughout the experiment by data loggers (Opus 10; Lufft, Fellbach, Germany). The temperature and humidity index (THI) values were calculated according to NRC (1971). Temperature and humidity during the P1 averaged 24 ± 2.4ºC and 68 ± 9%, respectively (THI = 72). During the P2, averages of temperature and humidity were 37.0 ± 1.8ºC and 49 ± 7% (THI = 87) during the day, and 31.0 ± 1.4ºC and 53 ± 7% (THI = 80) during the night.

Feed was offered once daily at 0930 h as a total mixed ration consisting of 85% alfalfa hay and 15% concentrate (as fed basis: oat grain 5%, malting barley 10%, canola meal 10%, gluten feed 10%, corn 4%, soy hulls 45%, soybean oil 5%, soybean meal 5%, molasses 2%, dicalcium phosphate 2.5%, salt 0.5%, premix 0.3%). Clean water was freely and individually available.

6.3.2. Experimental procedures

Performance. Feed intake and water consumption were recorded daily throughout the experiment. Feed refusals were collected and weighed at 0900 h in the morning and the amount of ration offered was adjusted to allow a refusal of 15-20%. Feed samples were collected at the beginning and end of the experiment for composition analyses. The BW was recorded weekly before feeding with a digital scale (Tru-Test AG500 Digital Indicator, accuracy, ± 20 g; Auckland, New Zealand). Growth rate was calculated as the difference between BW at the start and end of each week.

Physiological indicators. Rectal temperature (RT) and respiratory rate (RR) were recorded daily at 0800, 1200 and 1700 h throughout the experiment. The RT was recorded with a digital veterinary thermometer (model Accu-vet, ST714AC, range 32-42ºC; and accuracy ± 0.10ºC; Tecnovet S.L, Barcelona, Spain). The RR was calculated as the number of breaths per minute by counting the flank movements with the help of a chronograph and from a distance of 2 m without disturbing the goats.

Blood metabolites. Blood samples were collected from the jugular vein at day 3 of P1, and days 7 and 21 of P2 at 0800 h before feeding. Plasma was obtained by centrifugation for 15 min at 1500 × *g* and 4ºC and kept at –20°C for subsequent analysis of insulin, non-esterified fatty acids (NEFA), triglycerides, beta-hydroxy-butyrate (BHB), cholesterol, and glucose. Additionally, a second series of blood samples (0.3 mL) were collected using insulin syringes (1 mL, BD Micro-Fine, BD Medical-Diabetes Care, Franklin Lakes, NJ) for the instant analysis using an i-STAT System.

Metabolic tests. Four goats per treatment were subjected to 3 metabolic tests: glucose tolerance test (GTT), insulin tolerance test (ITT), and epinephrine challenge. One challenge per day was performed during 3 consecutive days (starting at days 4 and 12 of P1 and P2, respectively) in a randomized sequence and before the morning meal (0900 h) after 2 h fasting. For this purpose, a silicon catheter (Vygon, Ecouen, France) was inserted into the jugular vein the day before the first challenge for the administration of the glucose (0.25 g/kg BW), insulin (4.6 µg/kg BW), and epinephrine (2 µg/kg BW). Thereafter, 10 mL sterile solution was administered for flushing the catheter. Blood samples were collected into glass tubes, containing 250 IU of sodium heparin, and immediately placed on ice. The blood samples were collected at −20, −10, 0, 5, 10, 20, 30, 45, 60, 90, and 120 min relative to the administration of insulin, and epinephrine, whereas for glucose were collected until 90 min. After centrifugation of whole blood for 15 min at 1500 × *g* and 4°C, plasma was obtained and kept at –20°C for subsequent analysis of insulin, NEFA, BHB, and glucose concentrations according to the metabolic test.

6.3.3. Analytical procedures

Diet composition. Feed samples were ground through a 1-mm screen using a grinder machine, and dry matter (DM), acid-detergent fiber (ADF), neutral-detergent fiber (NDF) and ash content were analyzed according to the Association of Official Agricultural Chemists standard procedures (AOAC International, 2003). The content of crude protein (CP) was determined by the Dumas method (AOAC International, 2003) with a Leco Analyzer (Leco Corp., St. Joseph, MI) and calculated as N x 6.25. The chemical composition and nutritive value of the ration are shown in Table 6.1.

Blood metabolites and insulin. Insulin concentration was quantified with an ELISA kit (Mercodia Ovine Insulin ELISA; Mercodia, AD Bioinstruments, Terrassa, Spain). The NEFA were determined with a colorimetric enzymatic test ACS-ACOD method using a commercial kit (Wako Chemicals, Neuss, Germany). Triglycerides were analyzed with the glycerol-3-phosphate oxidase enzymatic method, and cholesterol was analyzed with the cholesterol esterase/peroxidase enzymatic method. Glucose was measured by the hexokinase method using an automatic analyzer system (Olympus AU400, Dusseldorf, Germany). BHB was determined by a kinetic enzymatic method using a commercial kit (Ranbut, Randox, UK).

Table 6.1. Chemical composition and nutritive value (dry-matter basis) of the ration.

Item	**Total mixed ration**
Component, %	
Dry matter	81.8
Organic matter	81.7
Crude protein	16.6
Neutral-detergent fiber	33.5
Acid-detergent fiber	23.2
Nutritive value[1]	
UEM, /kg	0.67
UFV, /kg	0.61
NEG, Mcal/kg	1.08
PDI, g/kg	94.7
PDIA, g/kg	54.3
RPB, g/kg	23.5
Ca_{abs}, g/kg	2.13
P_{abs}, g/kg	4.64

Legend: NEG net energy for growing; PDI protein digested in the small intestine supplied by microbial protein from rumen-fermented organic matter; PDIA protein digested in the small intestine supplied by rumen-undegraded dietary protein; RPB rumen protein balance; UFV forage unit for meat production.
[1] Calculated according to the Institute National de la Recherche Agronomique (INRA, 2018).

For the electrolyte, acid-base and metabolite analysis, blood samples were loaded into a blood gas cartridge (i-STAT EC8+ cartridge; Abbott Point of Care, Princeton, NJ) and immediately analyzed for major ions and metabolites: urea, creatinine, Cl, Na, K, iCa, total CO_2 concentration (TCO_2), anion gap (AnGap), hematocrit (Htc), hemoglobin (Hb) and were obtained.

Calculations and statistical analyses. To assess the response to the metabolic tests, the following parameters were calculated for blood metabolites and hormones (glucose, NEFA, BHB, insulin): basal concentration, peak or nadir concentration, area under curve (AUC) at 30, 45 or 90 min, and clearance rate (CR). Basal concentration, peak or nadir, and AUC were calculated from the actual values. The AUC between times was calculated using the trapezoidal method, in which the hormone or metabolite concentration was calculated by subtracting the actual value from the baseline value. The CR data were analyzed using the NLIN Procedure of SAS version 9.1.3 (Gauss-Newton method; SAS Institute Inc., Cary, NC) by fitting the exponential curves of glucose and insulin after the peak using the following equation (Hayirli et al., 2001):

$$F(t) = A * \exp^{(-k*t)}$$

Where,

F(t) is the concentration at time t.

A is the maximum concentration of the metabolite or insulin.

t is the time (min).

k is the regression coefficient.

The CR corresponds to the slope of the exponential function (*k*). The CR was calculated with the following equations:

$$CR\ (\%/\text{min}) = \{[\ln(t_a) - \ln(t_b)] \div (t_b - t_a)\} \times 100$$

Where,

t_a is the concentration of glucose or insulin at time a.

t_b is concentration of glucose or insulin at time b.

For the analysis of performance data (growth rate, DM intake, water consumption), physiological indicators (RT, RR), and blood metabolites and hormones, the PROC MIXED of SAS was used. The mixed model contained the fixed effects of treatment (IUTN vs. IUHS), period (P1 of TN vs. P2 of HS) and treatment × period interaction. Performance and physiological data were analyzed with the weekly average from daily databases. In addition, the hour effect was included (0800, 1200 or 1700 h) in the analysis of RT and RR.

Levels of NEFA, BHB, glucose, and insulin as responses to GTT, ITT, and epinephrine challenge in P1 and P2 were analyzed using PROC Mixed of SAS. The model contained the effects of treatment, time (min), and period and the treatment × time, treatment × period, period × time and treatment × period × time interactions as fixed effects. The calculated parameters (AUC, CR) were also pooled from P1 and P2 and analyzed by PROC Mixed using a model that contained the effects of treatment, period and treatment × period interaction. In all mixed models, animal was used as the random effect. Differences between least squares means were determined with the PDIFF test of SAS. Furthermore, with the CONTRAST statement of SAS the following orthogonal contrasts were tested: "P1 vs. P2", "IUTN vs. IUHS during P2", and "IUTN vs. IUHS during P1 vs. IUTN vs. IUHS during P2" (treatment × period interaction). Significance was declared at $P < 0.05$ and trend at $P < 0.10$ unless otherwise indicated.

6.4. Results

6.4.1. Physiological parameters

The effects of heat stress on rectal temperature (RT) at 0800, 1200 and 1700 h are presented in Figure 6.1. In utero TN or HS had no effect ($P = 0.798$) on RT response to HS at 8 months of age. Compared to P1, RT was increased ($P < 0.001$) in P2 with RT reaching the highest values at 1700 h (hour effect; $P < 0.001$). Furthermore, RT declined ($P < 0.001$) from week 1 to week 3 during the P2.

The IUHS had lower (94 vs. 105 breath/min; $P = 0.008$) RR throughout the day during P2 (HS) compared to IUTN goats. As shown in Figure 6.2, goats increased ($P < 0.001$) the RR immediately after the start of HS (P2) and then gradually decreased ($P < 0.01$) towards the end P2. Additionally, the RR was increased ($P < 0.05$) throughout the day from 0800 to 1700 h during P2 in accordance with the increment in ambient temperature from 30 to 37ºC.

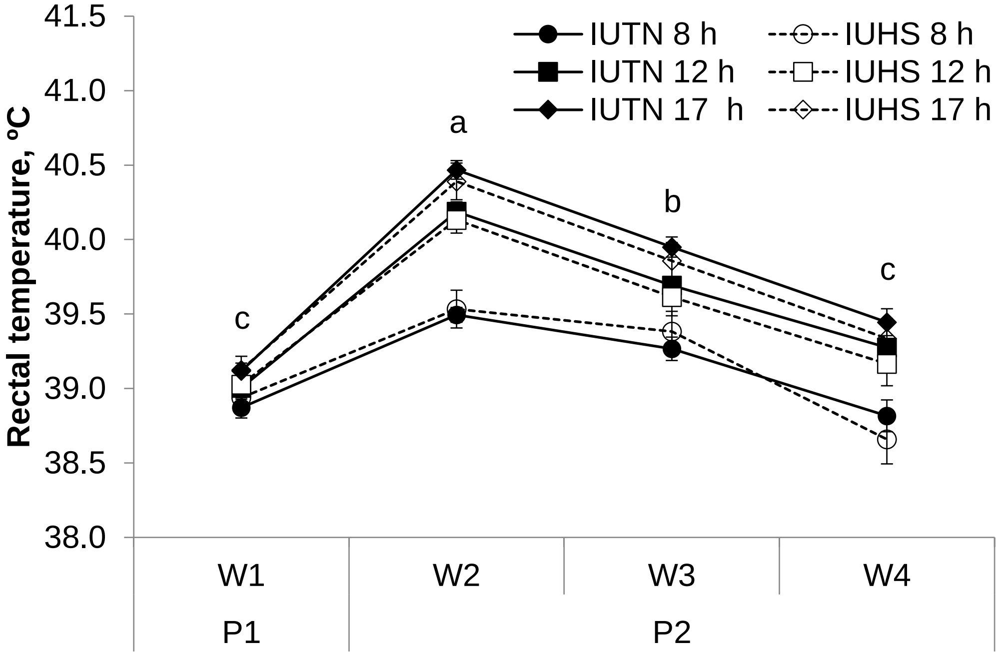


Figure 6.1. Rectal temperature at 8, 12 and 17 h in growing female goats that experienced in utero thermal-neutral (IUTN; n = 8) or heat stress (IUHS; n = 8) during the first 45 days of gestation. Both female-goat groups were exposed at 8 months of age to a period of TN (P1) for 1 week (W1) or to a period of HS (P2) for 3 weeks (W2 to W4). ^{a,b,c} Superscripts indicate significant difference (P < 0.05) comparing weeks throughout the experiment regardless the day hour.

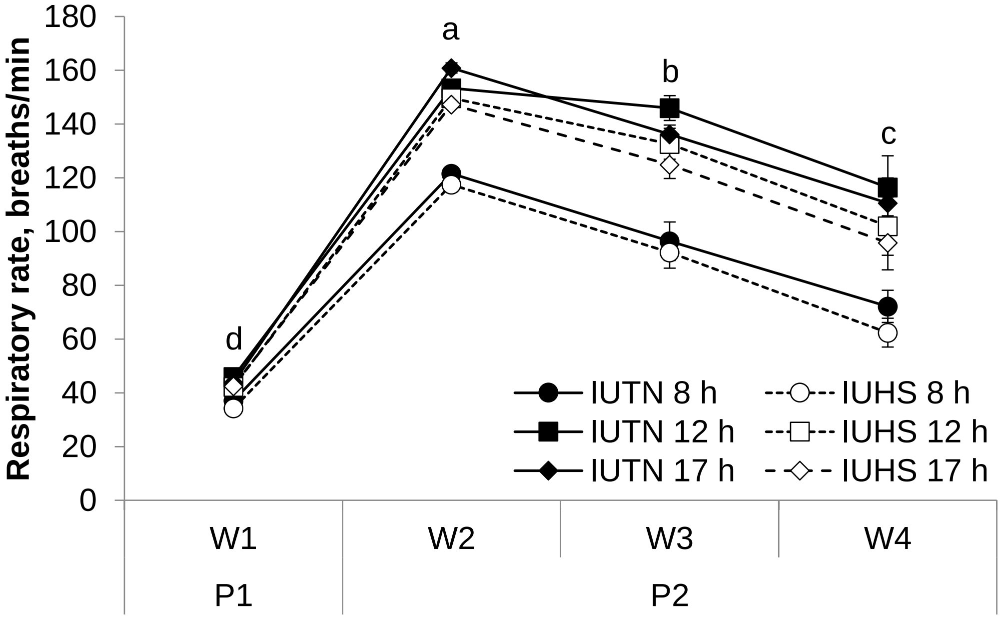


Figure 6.2. Respiration rate at 8, 12 and 17 h in growing female goats that experienced in utero thermal-neutral (IUTN; n = 8) or heat stress (IUHS; n = 8) during the first 45 days of gestation. Both female-goat groups were exposed at 8 months of age to a period of TN (P1) for 1 week (W1) or to a period of HS (P2) for 3 weeks (W2 to W4). ^{a,b,c,d} Superscripts indicate significant difference (P < 0.05) comparing weeks throughout the experiment regardless the day hour.

6.4.2. Productive variables

The average daily gain (ADG), feed intake and water consumption data are shown in Table 6.2. In utero HS did not affect ADG change ($P = 0.979$). However, BW increment in the growing goats during P2 (HS) was decreased ($P < 0.05$) by 56% compared to P1 (TN) on average. No treatment effect was observed on DMI ($P = 0.183$) or water intake ($P = 0.221$). Regardless the in utero thermal treatment, goats in P2 consumed 14% less ($P < 0.05$) DM intake and drank 67% greater ($P < 0.001$) water volume than in P1 (TN). Within the P2 (the HS period), DM intake and water consumption declined ($P < 0.05$) from week 1 to 3.

Table 6.2. Change in body weight, DM intake, and water consumption of growing female goats that experienced *in utero* thermal-neutral (IUTN; n = 8) or heat stress (IUHS; n = 8) during the first 45 days of gestation. Both female-goat groups were exposed at 8 months of age to a period of TN (P1) for 1 week (W1) or to a period of HS (P2) for 3 weeks (W2 to W4).

Item	P1	P2			SEM¹	Effect¹ (*P* <)		
	W1	**W2**	**W3**	**W4**		**T**	**P**	**T × P**
ADG, g/d	49.2^{a}	25.0^{b}	26.0^{ab}	14.5^{b}	8.9	0.979	0.012	0.675
DMI, kg/d	0.71^{a}	0.71^{a}	0.61^{b}	0.53^{c}	0.04	0.183	0.011	0.751
G:F	0.07^{a}	0.04^{b}	0.04^{ab}	-0.02^{b}	0.05	0.718	0.011	0.567
WI, L/d	1.31^{c}	2.44^{a}	2.26^{a}	1.85^{b}	0.05	0.221	0.001	0.352

Legend: ADG: average daily gain; DMI: dry matter; G:F gain-to-feed ratio; WI: water intake.

[1] Standard error of the mean.

[2] Effects of in utero thermal treatment (T), period (P) and their interaction (T × P).

[a,b,c] Means of weeks throughout the experiment with different superscripts differ ($P < 0.05$).

6.4.3. Blood metabolites and insulin

Values of blood metabolites and insulin in goats that experienced or not in utero HS are shown in Figure 6.3. No differences ($P > 0.55$) between IUTN and IUHS were observed through the experiment in any of the metabolites measured (glucose, NEFA, BHB, cholesterol, albumin). Shifting from P1 (TN) to P2 (HS) had no effect on blood metabolites or insulin, except glucose that was reduced ($P = 0.012$) in the P2. Nonetheless, a week effect ($P < 0.05$) was observed for cholesterol, NEFA, albumin and glucose, where albumin and glucose decreased while NEFA and cholesterol increased over time. In the case of insulin, IUTN animals had greater ($P < 0.05$) concentrations during week 1 of P2 (HS), and in week 3 hey returned to the P1 (TN) levels. On the other hand, basal insulin levels in IUHS goats did not vary throughout P1 and P2.

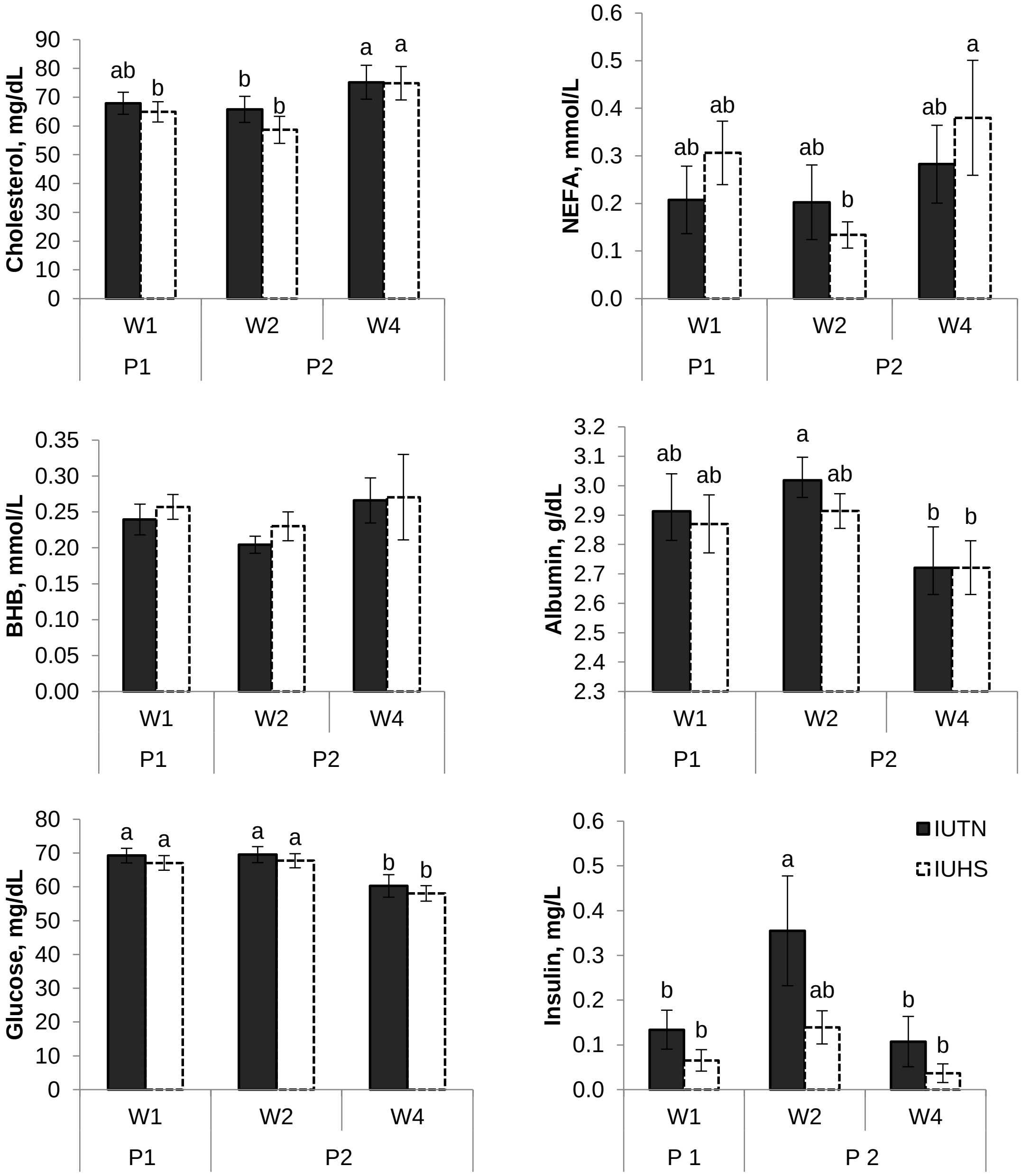


Figure 6.3. Blood cholesterol, NEFA, BHB, albumin, glucose, and insulin in growing female goats that experienced in utero thermal-neutral (IUTN; n = 8) or heat stress (IUHS; n = 8) during the first 45 days of gestation. Both female-goat groups were exposed at 8 months of age to a period of TN (P1) for 1 week (W1) or to a period of HS (P2) for 3 weeks (W2 to W4). ^{a,b} Superscripts indicate significant difference ($P < 0.05$) comparing weeks throughout the experiment. Blood samples were collected in the morning before feeding when ambient temperatures were at lowest values.

Metabolic and acid-base balance indicators are shown in Table 6.3. In utero HS or TN had no effect ($P > 0.10$) on any of the metabolic variables. The HS in P2 increased ($P < 0.01$) blood values of Na, TCO_2, and anion gap. Blood Cl, on the other hand, tended to decrease ($P <$

0.06) during P2. There was week effect ($P < 0.05$) during P2, where Na, TCO_2 and hematocrit increased, whereas Cl decreased throughout P2. Additionally, there was a transit ($P < 0.05$) increase and decrease in iCa and creatinine, respectively during week 1 of P2, but values returned to the TN values (P1) by week 3. Treatment by period interaction was detected ($P < 0.001$) for blood urea as IUHS animals had greater basal values compared to IUTN in P1 (TN), but during P2 (HS) blood urea tended to be greater ($P < 0.01$) for IUTN goats at week 3 (Figure 6.4).

Table 6.3. Metabolic and acid-base indicators in growing female goats that experienced in utero thermal-neutral (TN; n = 8) or heat stress (HS; n = 8) during the first 45 days of gestation. Both female-goat groups were exposed at 8 months of age to a period of TN (P1) for 1 week (W1) or to a period of HS (P2) for 3 weeks (W2 to W4). Blood samples were taken in the morning before feeding when ambient temperatures were at lowest values. Values are least square means and standard error of the mean (SEM).

Item	P1	P2		SEM[1]	Effect[2] (P <)		
	W1	**W2**	**W4**		**P**	**T at P2**	**T × P**
BUN	20.9	22.1	21.3	0.92	0.363	0.113	0.001
Creatinine	0.66^a	0.53^b	0.65^a	0.03	0.085	0.903	0.813
Na, mmol/L	142^b	143^b	146^a	0.43	0.004	0.801	0.959
K, mmol/L	4.25	4.21	4.08	0.09	0.168	0.181	0.221
Cl, mmol/L	105^a	105^a	102^b	0.64	0.055	0.249	0.788
iCa, mmol/L	1.30^b	1.33^a	1.29^b	0.01	0.422	0.178	0.663
TCO_2, mmHg	23.0^b	21.8^b	27.1^a	0.64	0.010	1.000	0.813
Htc, %	19.9^b	18.5^c	21.5^a	0.70	0.893	0.821	0.501
Hb, g/dL	6.78^a	6.32^b	7.00^{ab}	0.34	0.635	0.539	0.306
AnGap	19.9^b	21.4^a	21.5^a	0.25	0.001	0.131	0.940

Legend: BUN: blood urea nitrogen; Htc: hematocrit; Hb: hemoglobin.

[1] Standard error of the mean.

[2] IUHS vs. IUTN during HS period (T at P2), period (P) and the interaction Effects between in utero thermal treatment and period (T × P).

[a,b,c] Means of weeks throughout the experiment with different superscripts differ ($P < 0.05$).

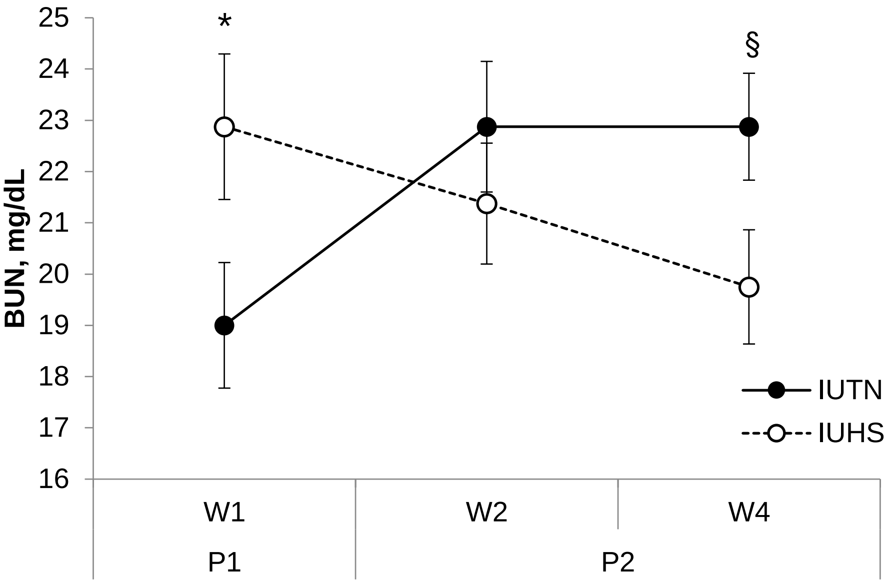


Figure 6.4. BUN concentrations in growing female goats that experienced in utero thermal-neutral (IUTN; n = 8) or heat stress (IUHS; n = 8) during the first 45 days of gestation. Both female-goat groups were exposed at 8 months of age to a period of TN (P1) for 1 week (W1) or to a period of HS (P2) for 3 weeks (W2 to W4). * Means differ between treatments ($P < 0.05$). § Means tend to differ between treatments ($P < 0.10$).

6.4.4. Responses to the metabolic tests

Glucose, insulin, and NEFA responses to the GTT during P1 and P2 are shown in Table 6.4 and Figure 6.5. Five min after glucose administration, blood glucose reached its maximum concentration and returned to the basal levels by 30 min. In utero thermal treatment had no effect on glucose kinetics, except for AUC that tended to be greater ($P < 0.10$) in IUHS compared to IUTN animals. On the other hand, shifting from P1 (TN) to P2 (HS) resulted in increased ($P < 0.05$) basal glucose level, but glucose peak tended to decrease ($P < 0.10$). The IUHS goats had lower ($P < 0.05$) insulin clearance rate in response to GTT (Table 6.4). Additionally, HS increased ($P = 0.035$) the basal levels of insulin regardless the in utero thermal treatment. In utero thermal stress did not affect blood values of NEFA in the GTT (Table 6.4). However, basal levels of NEFA and AUC at 45 min tended to increase ($P < 0.10$) in P2 (HS) compared to P1 (TN). No treatment by period interaction was detected for all metabolite pharmacokinetics (Table 6.4).

Table 6.4. Glucose, insulin, and NEFA responses to glucose tolerance test in growing female goats that experienced in utero thermal-neutral (IUTN; n = 4) or heat stress (IUHS; n = 4) during the first 45 days of gestation. Both goat groups were exposed at 8 months of age to a period of TN (P1) for 1 week or to a period of HS (P2) for 3 weeks.

Item	IUTN		IUHS		SEM[1]	Effect[2] (*P* <)		
	P1	P2	P1	P2		T	P	T × P
Glucose								
Basal, mg/dL	59.3	67.2	62.1	66.4	2.3	0.776	0.017	0.381
Peak, mg/dL	580	359	612	434	126	0.773	0.073	0.824
CR_{5-45}, %/min	15.4	7.8	11.0	9.6	4.9	0.858	0.239	0.397
AUC, mg/dL×min								
45 min	5,927	6,114	7,988	6,843	652	0.077	0.274	0.145
90 min	8,468	9,335	11,074	10,266	530	0.056	0.964	0.243
Insulin								
Basal, µg/L	0.13	0.15	0.12	0.24	0.03	0.409	0.035	0.135
Peak, µg/L	2.40	2.50	2.00	2.81	0.38	0.929	0.319	0.431
CR_{10-60}, %/min	5.13	4.74	2.67	3.32	0.54	0.043	0.746	0.207
AUC µg/L×min								
45 min	51.1	54.0	52.3	73.7	7.97	0.388	0.279	0.400
90 min	56.5	62.0	64.2	91.5	9.20	0.203	0.254	0.434
NEFA								
Basal, mmol/L	0.116	0.318	0.230	0.283	0.052	0.605	0.059	0.226
Peak, mmol/L	0.250	0.398	0.360	0.443	0.053	0.349	0.225	0.729
AUC, mmol/L×min								
45 min	6.72	12.0	7.91	13.6	2.0	0.640	0.060	0.927
90 min	11.8	22.0	13.8	21.2	3.2	0.892	0.108	0.773

Legend: AUC = area under the curve; CR = clearance rate; NEFA = non-esterified fatty acids.

[1] Standard error of the mean.

[2] Effects of in utero thermal treatment (T), period (P) and their interaction (T × P).

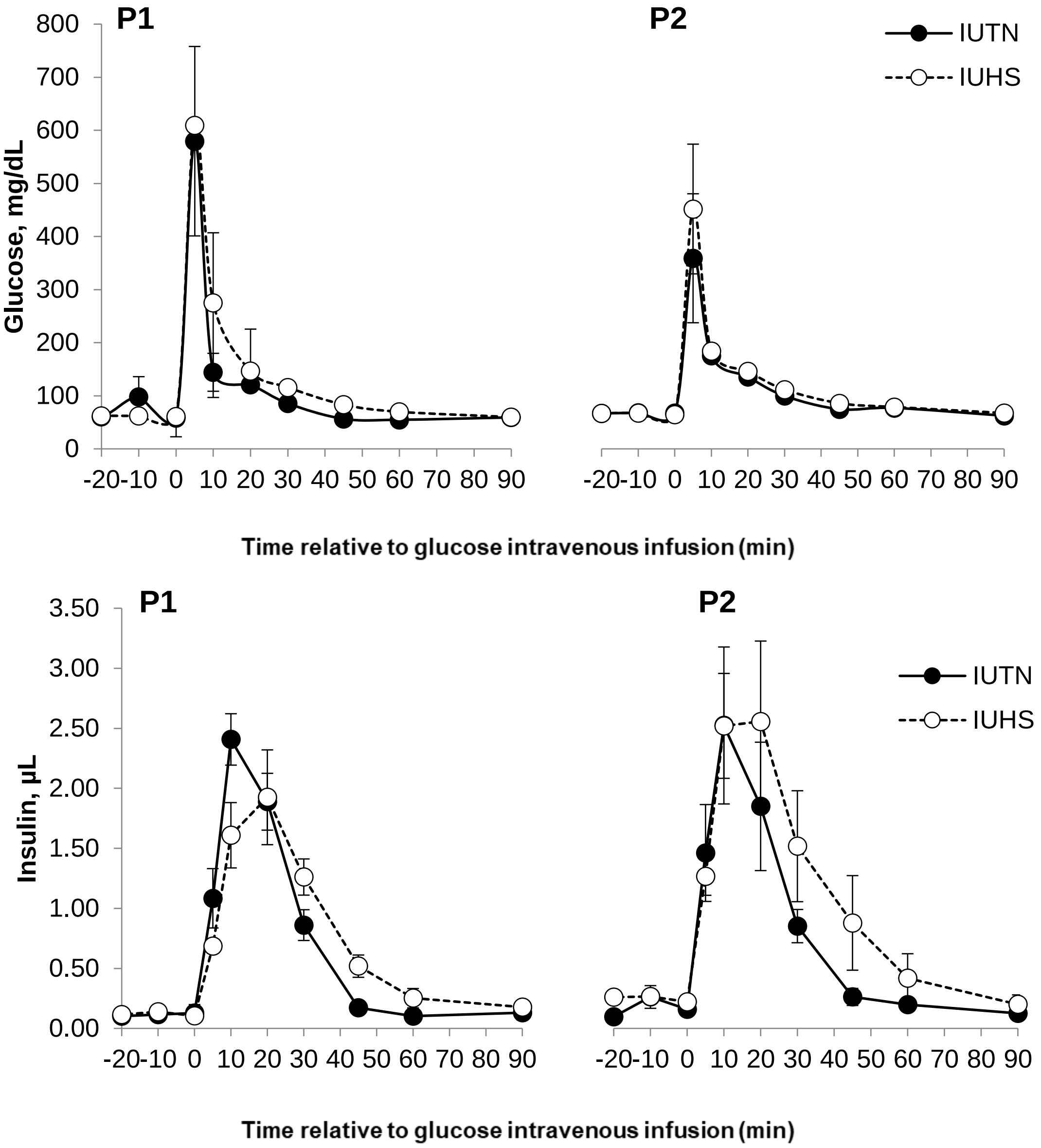


Figure 6.5. Glucose and insulin responses to glucose tolerance test (GTT) in growing female goats that experienced in utero thermal-neutral (IUTN; n = 4) or heat stress (IUHS; n = 4) during the first 45 days of gestation. Both goat groups were exposed at 8 months of age to a period of TN (P1) for 1 week or to a period of HS (P2) for 3 weeks. The GTT was carried out during week 1 and week 2 for P1 and P2, respectively. Vertical bars indicate SE.

With regard to the ITT, neither in utero thermal treatment nor HS at 8 months of age affected the glucose pharmacokinetic response (Table 6.5). As shown in Figure 6.6, glucose levels were immediately decreased after insulin administration, reaching the lowest values ($P < 0.001$) at 30 min and returning to basal values at 120 min in IUTN but not in IUHS goats. In addition, during the P2 (HS) IUTN had greater ($P < 0.05$) blood glucose at 45 and 120 min after IC compared to IUHS goats (significant interaction between treatment and time relative to ITT; $P < 0.001$). In contrary to what observed for the basal NEFA levels in GTT (Table 6.4), the basal NEFA levels were lower ($P < 0.001$) during P2 (HS) than P1 (TN) as shown in Table 6.5. Furthermore, there was a NEFA peak at 45 min after ITT (Figure 6.6), and this peak tended to be lower ($P = 0.108$) during P2 than P1. The AUC at 120 min also tended to be lower ($P = 0.077$) during P2 than P1 (Table 6.5).

Table 6.5. Glucose and NEFA responses to insulin tolerance test in growing female goats that experienced in utero thermal-neutral (IUTN; n = 4) or heat stress (IUHS; n = 4) during the first 45 days of gestation. Both goat groups were exposed at 8 months of age to a period of TN (P1) for 1 week or to a period of HS (P2) for 3 weeks.

Item	**IUTN**		**IUHS**		**SEM**[1]	**Effect**[2] **(*P* <)**		
	P1	**P2**	**P1**	**P2**		**T**	**P**	**T × P**[1]
Glucose								
Basal, mg/dL	66.2	67.8	66.0	64.2	2.7	0.639	0.939	0.425
Nadir, mg/dL	20.2	26.3	29.8	29.0	2.6	0.123	0.490	0.375
$CR_{0\text{-}30}$, %/min	3.97	3.47	2.87	2.67	0.36	0.116	0.354	0.669
AUC mg/dL × min								
30 min	1,221	1,244	1,357	1,335	692	0.288	0.989	0.761
120 min	5,756	6,513	5,685	5,888	386	0.548	0.215	0.453
NEFA								
Basal, mmol/L	0.473	0.250	0.588	0.285	0.044	0.277	0.001	0.411
Peak, mmol/L	0.745	0.558	0.755	0.610	0.071	0.766	0.109	0.818
AUC mmol/L × min								
120 min	49.0	38.8	57.8	46.8	5.29	0.306	0.077	0.929

Legend: AUC: area under the curve; CR: clearance rate; NEFA: non-esterified fatty acids.

[1] Standard error of the mean.

[2] Effects of in utero thermal treatment (T), period (P) and their interaction (T × P).

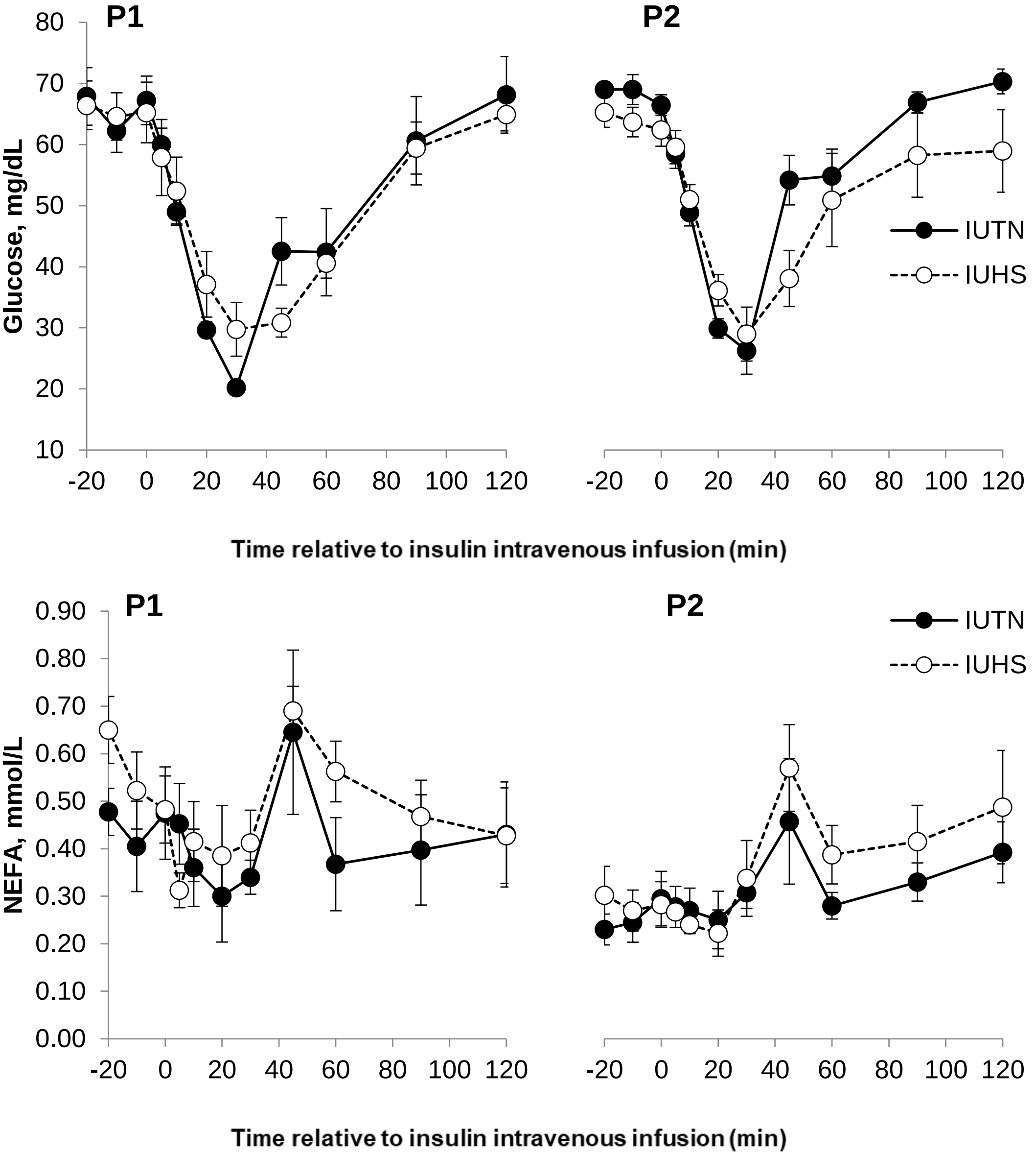


Figure 6.6. Glucose and NEFA responses to insulin tolerance test (ITT) in growing female goats that experienced in utero thermal-neutral (IUTN; n = 4) or heat stress (IUHS; n = 4) during the first 45 days of gestation. Both female-goat groups were exposed at 8 months of age to a period of TN (P1) for 1 week or to a period of HS (P2) for 3 weeks. The ITT was carried out during week 1 and week 2 for P1 and P2, respectively. Vertical bars indicate SE.

In utero thermal treatment and HS at 8 months of age had no effect on glucose pharmacokinetic responses to the epinephrine challenge (Table 6.6). Epinephrine administration triggered a peak ($P < 0.001$) in blood glucose at 5 min that gradually decreased afterwards (Figure 6.7). Compared to the basal values, there was no clear peak of NEFA after epinephrine administration, despite the fact that epinephrine is a lipolytic signal. Compared to P1 (TN), HS during P2 increased the AUC at 120 min ($P < 0.001$) to a greater extent in IUTN goats compared to IUHS ones (significant treatment by period interaction; Table 6.6).

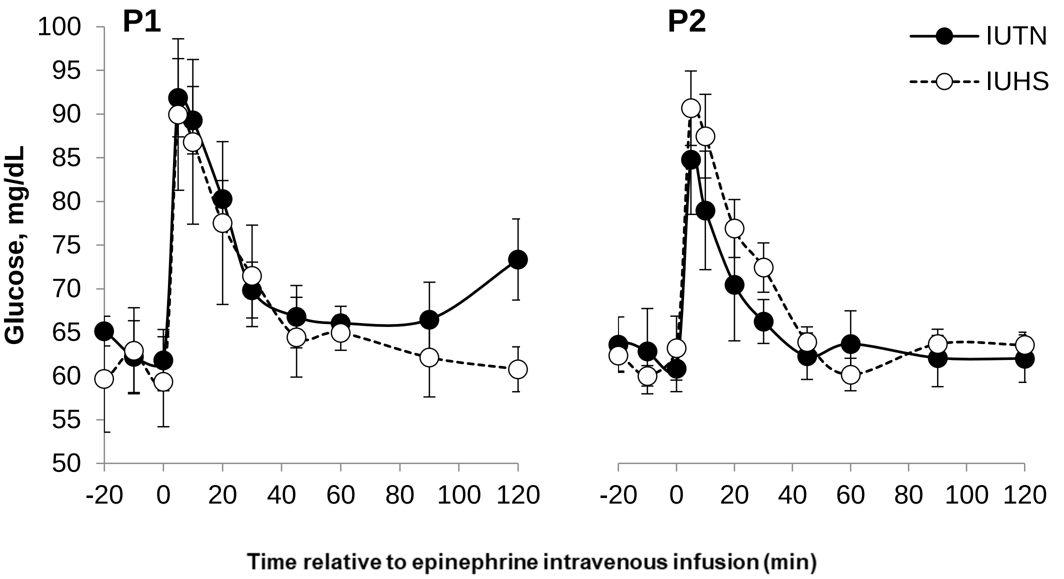
Table 6.6. Glucose and NEFA responses to epinephrine challenge in growing female goats that experienced in utero thermal-neutral (IUTN; n = 4) or heat stress (IUHS; n = 4) during the first 45 days of gestation. Both goat groups were exposed at 8 months of age to a period of TN (P1) for 1 week or to a period of HS (P2) for 3 weeks.

Item	IUTN		IUHS		SEM[1]	Effect[2] (*P* <)		
	P1	**P2**	**P1**	**P2**		**T**	**P**	**T × P[1]**
Glucose								
Basal, mg/dL	64.0	61.6	60.7	61.9	3.4	0.765	0.787	0.438
Peak, mg/dL	94.6	86.7	91.6	91.7	3.7	0.849	0.608	0.597
$CR_{5\text{-}45}$, %/min	0.89	0.78	0.85	0.89	0.10	0.806	0.775	0.589
AUC, mg/dL × min								
45 min	3,465	3,161	3,394	3,409	124	0.610	0.554	0.491
120 min	8,520	7,849	8,113	8,518	254	0.829	0.375	0.372
NEFA								
Basal, mmol/L	0.282	0.538	0.350	0.318	0.07	0.399	0.127	0.062
Peak, mmol/L	0.470	0.718	0.435	0.470	0.07	0.173	0.095	0.179
AUC mmol/L × min								
120 min	30.8	66.8	35.5	41.9	5.3	0.225	0.009	0.039

Legend: AUC: area under the curve; CR: clearance rate; NEFA: non-esterified fatty acids.

[1] Standard error of the mean.

[2] Effects of in utero thermal treatment (T), period (P) and their interaction (T × P).



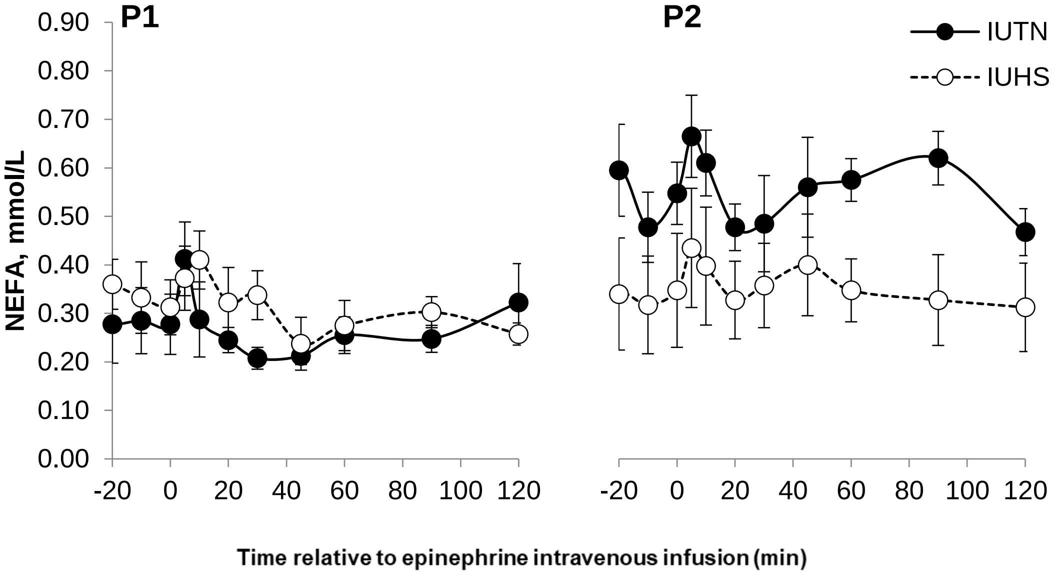


Figure 6.7. Glucose and NEFA responses to epinephrine challenge in growing female goats that experienced in utero thermal-neutral (IUTN; n = 4) or heat stress (IUHS; n = 4) during the first 45 days of gestation. Both female-goat groups were exposed at 8 months of age to a period of TN (P1) for 1 week or to a period of HS (P2) for 3 weeks. The epinephrine challenge was carried out during week 1 and week 2 for P1 and P2, respectively. Vertical bars indicate SE.

6.5. Discussion

Heat stress has been studied with special interest in dairy animals because of its negative impact on milk production, reproduction, health, and welfare (West, 2003). With regard to goats, most of published research focused on the impact of HS on adult animals, especially dairy breeds (Sano et al., 1985; Brown et al., 1988; Hirayama and Katoh, 2004; Hamzaoui et al., 2013; Salama et al., 2014; Contreras-Jodar et al., 2018; Mehaba et al., 2019). Little is known regarding the performance and physiological responses of young growing goats to heat stress. The age and its related factors (body composition, hormonal milieu, nutritional partitioning, etc.) would result in different response to HS compared to adult dairy animal. Additionally, no data are available on the physiological mechanisms that underlie the responses to in utero heat stress later in life in goats. Metabolic dysfunction in the offspring has been reported due to prenatal insults in human (Roseboom et al., 2006), sheep (Limesand et al., 2007), pigs (Johnson et al., 2015a), and rats (Knittle and Hirsch, 1968). Herein we report the metabolic responses to *in utero* HS in the goat specie.

Both IUTN and IUHS goats exhibited increased RT and RR during the heat exposure period (P2) compared to the TN period (P1), which agrees with results observed in adult goats (Hamzaoui et al., 2013; Castro-Costa et al., 2015; Shilja et al., 2016; Al-Dawood, 2017). Homoeothermic animals regulate their body temperature by the balance between the amount of heat produced coming from the body and metabolism, and the heat the animal emits towards the environment (Hansen, 2004). Therefore, greater RR helps animals to dissipate the extra heat. Values of total blood CO_2 numerically decreased during the first week of HS and then increased by week 3 of the HS period. The increment in blood CO_2 during HS is unexpected because it typically is reduced in adult goats under HS conditions due to panting (Sivakumar et al., 2010; Hamzaoui et al., 2013). Independent of the in utero thermal treatment, growing goats experienced a reduction in RT and RR throughout the 3-week HS period, which is likely an indication of HS acclimation.

Our IUHS goats needed 10% less RR in order to keep similar RT compared to IUTN goats during the HS exposure period. Ahmed et al. (2017) reported that cows born to mothers exposed to high ambient temperatures during last 2 months of gestation have reduced elevations in rectal temperature, sweating rate, and increased skin temperature when they experienced acute heat stress later in life. Those authors concluded that in utero HS cows appear to dissipate heat more efficiently than in utero control cows because the increased skin temperature in in utero HS cows could be related to more skin blood flow. On the contrary, pigs that were heat-stressed in utero during the first or second half of gestation have increased core body temperatures during adult life (Johnson et al., 2015b), suggesting that in utero HS does not alter thermotolerance later in life in pigs.

The fact that our IUHS goats respired less but kept similar body temperature might indicate that they were able to loss heat by alternative ways rather than panting. It is possible that they had greater skin temperature associated with increased blood flow to the skin, and consequently greater cooling via conduction as suggested by Ahmed et al. (2017). In fact, an increment in skin temperature is an indicator of enhanced body heat dissipation (Blatteis, 1998). Alternatively, our IUHS would have been able to sweat more compared to the control animals, and consequently they dissipated more heat by evaporation from skin rather than from lungs by panting. Sweating accounts for 70 to 85% of heat loss via evaporation, whereas respiration is responsible for the remainder 15 to 30% (Finch, 1986). Unfortunately, we did not measure skin sweating rate or skin temperature to confirm these assumptions.

Growing goats under HS in the present study ate less (-14% on average), but were still able to gain BW under HS, although to lower extent (15 g/d), compared to TN (49 g/d). Similarly, Ames and Brink (1977) reported that lambs at 4 months age exhibit less BW gain when moved from 20ºC (184 g/d) to 35ºC (41 g/d). On the other hand, lactating dairy goats (Hamzaoui et al., 2013; Contreras-Jodar et al., 2018; Mehaba et al., 2019) eat 21 to 35% less and loss 10 to 146 g/d BW. The reduction in growth rate during P2 (HS) is obviously due to the decreased DM intake, but also could partially stem from the increased maintenance requirements by HS. Consequently, lesser nutrients are available for growth. Numerous studies suggest that heat stress exposure increases maintenance costs by as much as 25% in sheep (Ames et al., 1971), goats (Salama et al., 2014), and cows (Fox and Tylutki, 1998). Thus, our HS goats experienced reduced feed intake and in the same time increased maintenance requirements necessary for extra activities that include 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 (Salama et al., 2014).

In the present study, blood glucose concentration in both goat groups decreased during the last week of heat exposure. This finding contradicts the results observed in lactating dairy goats (Hamzaoui et al., 2013; Mehaba et al., 2019) and dry goats (Castro-Costa et al., 2015), where blood glucose is not affected by HS. Reduced glucose under heat stress has also been observed in dairy cows, growing steers, and sheep (Achmadi et al., 1993; Settivari et al., 2007; O'Brien et al., 2010; Gonzalez-Rivas et al., 2017). Altered physiological states (lactation vs. growth) may contribute to the variation observed in circulating glucose concentrations between growing and adult goats.

There was a transient increase in basal blood insulin during week 1 of HS (acute response to HS) in IUTN goats, but not in IUHS goats. The increased basal insulin concentration in response to HS is consistent with findings in rodents (Torlińska et al., 1987), pigs (Hall et al., 1980), and dairy cows (Wheelock et al., 2010), but not in lactating dairy goats that did not experienced any change in blood insulin under HS conditions (Hamzaoui et al., 2013). The increased insulin may be an essential part of the adaptation mechanism to HS that was needed in IUTN goats but was not necessary in the IUHS animals.

Plasma NEFA, BHB and cholesterol did not differ by in utero thermal treatment or HS exposure at 8 months of age. These findings agree with what observed in lactating dairy goats exposed to HS (Sano et al., 1985; Hamzaoui et al., 2013; Mehaba et al., 2019), despite the reduced feed intake. The BUN was greater in IUHS than IUTN during P1 (TN) and vice versa during the week 3 of P2 (HS). It is not clear why IUTH and IUHS behaved this way with regard to BUN. The BUN is related to protein intake level, efficiency of rumen microbial N incorporation into microbial protein, and skeletal muscle proteolysis. Feed intake (and consequently ingested protein) did not vary between IUTH and IUHS throughout the experiment. Additionally, blood creatinine level (indicator of muscle degradation) was similar between both goat groups during all weeks. During the P2, the HS-induced increase in BUN in IUTN may stem from inefficient rumen microbial N incorporation, as HS is thought to alter rumen fermentation patterns. In a previous study, we observed lower rumen pH in HS goats compared to TN goats, despite the fact that both goat groups fed the same amount of food (Castro-Costa et al., 2015). Whether in utero HS would affect rumen fermentation under hot conditions deserves more research.

To gain a better understanding of the changes in metabolism, we performed GTT, ITT and epinephrine challenge during the TN period (P1) and repeated them during the HS period (P2). Carrying out these metabolic tests during the 2nd week of P2 was after the acute response to HS during week 1. We chose the 2nd week as the middle point in the HS period. Glucose clearance rate in response to GTT did not vary between IUTN and IUHS goats or between TN and HS periods. Despite a lack of difference in plasma glucose clearance to the GTT, IUHS goats displayed a lower insulin clearance rate.

Following the glucose infusion, peak glucose concentration tended to be lower in P2 (HS) than in P1 (TN), most likely due to a greater glucose requirements and usage as indicated above. The rate of glucose disappearance in response to insulin administration is a sensitive measure of acute insulin action. When insulin was administered during P2 (HS), its effect on lowering blood glucose was more prolonged in IUHS goats (lower blood glucose levels from 45 to 90 min after ITT compared to IUTN goats. This result was in accordance with the lower clearance rate of insulin in IUHS animals as indicated by the GTT. Additionally, insulin secretion in response to GTT was greater during P2 in IUHS compared to IUTN goats. Together, these findings could indicate that under HS conditions the pancreas of IUHS became more sensitive to glucose (secreting more insulin to the same dose of glucose), and the secreted insulin stayed for longer time. On the other hand, the glucogenic response to epinephrine did not vary by in utero thermal treatment (IUTN vs. IUHS) or period (P1 vs. P2).

Similar to our results in young goats, lambs born to ewes exposed to HS during mid-gestation exhibited enhanced insulin response to glucose administration (Yates et al., 2011). In contrast, pancreas sensitivity to glucose stimulation did not vary between calves born to cows suffering HS or cooled during the last 2 months of gestation (Tao et al., 2014). The discrepancy between studies might be explained by the stage of gestation at which HS was applied. Fetal pancreas development in ruminants occurs during the first 2 trimesters of gestation and remains steady thereafter (Guilloteau et al., 2009). Thus, HS during early gestation (current study in goats) and mid-gestation (Yates et al., 2011, in sheep) may have more impact on pancreatic development compared to what observed in bovine at late gestation (Tao et al., 2014).

The increased insulin secretion of IUHS under HS conditions, but not in TN environment, may suggest a possibility that goat-kids born to heat-stressed dams during gestation are more prone to develop fatter body in the postnatal life if raised in hot conditions compared with IUTN goat-kids. Published research indicates similar outcome as low-birth-weight lambs born to dams experiencing induced placental restriction have compensatory growth with more body fat after birth compared with normal lambs (De Blasio et al., 2007). Similarly, embolization of placental vasculature during gestation results in negative effects on fetal growth and postnatal lamb body composition (Louey et al., 2005).

Insulin has a lipogenic effect, whereas epinephrine is a lipolytic signal (Hayirli, 2006) and when administered blood NEFA should decrease and increase, respectively. Epinephrine binds to the β-adrenergic receptors on adipocytes, resulting in the release of NEFA in the circulation. However, and regardless the in utero thermal stress treatment, we did not detect changes in blood NEFA levels following ITT or epinephrine challenge compared to the basal levels, despite the fact that insulin and epinephrine doses used in the current study were enough to induce clear changes in blood glucose levels. This contradicts the obtained results in adult cows (Tao et al., 2014) and goats (Salama et al., 2014), where the typical effects of insulin and epinephrine on blood NEFA were observed. We should keep in mind that goats in the current study were young with presumably lower fat depots compared to adult animals. This might explain the no fat mobilization in response to epinephrine stimulation. Resende et al. (2017) reported that body fat content increased from 5 to 13% for goat kids weighing 5 to 26 kg, respectively (age of 0.5 to 9 months). Alternatively, adipose tissue of young goats could have less response to epinephrine compared to the adult animals. In fact, Martin et al. (2011) reported that NEFA response to adrenaline increases with age from 4 to 16 months of age in sheep.

Independent of in utero thermal treatment, the AUC of NEFA in repose to ITT tended to decrease during P2 (HS period) compared to P1 (TN). This agrees with results observed in adult dairy goats that mobilize less body fat under HS conditions (Salama et al., 2014). For the epinephrine challenge, the AUC of NEFA in IUTN goats increased by HS, but IUHS goats did not experience such an increase when exposed to HS. This might indicate that lipid tissue in IUHS goats became more resistant to lipolytic signals under HS conditions compared to IUTN goats. In fact, heat-stressed dairy cows (Rhoads et al., 2009) and goats (Salama et al. 2014) mobilize significantly less body fat in response to epinephrine challenge compared to dairy goats maintained under thermal-neutral conditions.

6.6. Conclusions

Independent of in utero thermal treatment during the first 45 days of gestation, typical responses to heat stress (reduced feed intake and growth rate, and increased DM intake, water consumption, rectal temperature and respiratory rate) were observed when female 8-months of age goats were exposed to high ambient temperature. However, IUHS goats needed less overall breaths/min to keep similar rectal temperatures to IUTN goats, indicating that they might be able to loss heat by alternative ways rather than panting. Additionally, the pancreas of IUHS goats became more sensitive to glucose under HS conditions and the secreted insulin stayed for longer time, which may suggest IUHS kids might be more prone to have fatter body in the postnatal life if raised in hot conditions. This would have impact on the future productive life as increasing body fat in growing dairy animals is known to reduce milk production.

CHAPTER 7

Conclusions and Implications

The conclusions derived from the different experiments included in the present PhD thesis are the following:

Response of dairy goats to cold stress:

1. Low temperatures ranging between -3 to 6ºC have a direct effect on the physiological parameters of dairy goats such that respiration rate and rectal temperature are reduced (−6 breaths/min; −0.32°C, respectively).
2. Low ambient temperatures reduce milk production on the 3rd week of exposure and increase the milk component contents in dairy goats.
3. Low temperatures do not alter their feed intake, but elicit a reduction in the body weight (−0.45 kg) compared to thermal-neutral conditions.
4. Low temperatures induce lipid reserves mobilization as evidenced by greater blood NEFA concentrations than in thermo-neutral temperatures. However, the increment in NEFA is not accompanied by greater blood levels of BHB.
5. Low ambient temperatures do not modify blood insulin concentrations; however, goats under low ambient temperatures show greater values of blood glucose than thermo-neutral ones.

Prenatal heat stress effects at parturition and on behavior of goat kids:

6. Heat stress during the early gestation period increases the rectal temperature and respiration rate (+0.68ºC, and +76 breaths/min, respectively) and decreases feed intake by 15% in the dairy pregnant goats.
7. Heat stress from mating until the first 45 d of gestation of dairy goats shortens the duration of pregnancy by 3 days.
8. The behavioral response of kid goats to novel environment and object is altered by *in utero* heat stress, as evidenced by a reduction in the number of sniffing events.
9. Drinking behavior increases dramatically in growing female goats at the beginning of heat exposure and declines throughout the experimental period.

10. Behavioral parameters such as feeding, exploration and grooming are reduced after challenging growing female goats with heat-stress. However, the in uterus thermal treatment do not alter these responses.
11. Postural behaviors are also altered by heat exposure with increases in the frequency of lying.

Prenatal heat stress effects on performance and metabolism of goats exposed to heat stress at 8 months of age.

12. Independent of in utero thermal stress, growing goats consume less feed (-14% on average) and acheive less body weight gain compared to thermo-neutral conditions.
13. Growing goats exposed to in utero heat stress respire less (94 vs. 105 breaths/min; $P = 0.008$), but are able to keep similar body temperature compared to in utero thermal neutral ones.
14. Growing goats that did not experience in utero heat stress show a transient (acute heat stress response) increase in basal concentrations of circulating insulin when exposed to postnatal heat challenge, whereas in utero heat-stressed goats do not show such an acute insulin response.
15. In utero heat-stressed growing goats infused with glucose display greater insulin secretion and this secreted insulin stays in the circulation for longer time.

Implications:

- Goats are sensitive to low ambient temperatures as their milk yield is decreased after 3 weeks of exposure to cold temperatures, and body fat reserves are mobilized to cover the extra heat production. Thus, goats should not be exposed to ambient temperatures below zero.
- Heat stress during pregnancy should be avoided as it modifies behavior during the postnatal life. Additionally, it may result in fatter body if goats are raised in hot conditions. This greater body fat would reduce mammary secretory tissue and might cause decreased milk yield in the future productive life.

CHAPTER 8

References

Achmadi, J., T. Yanagisawa, H. Sano, and Y. Terashima. 1993. Pancreatic Insulin Secretory Response and Insulin Action in Heat-Exposed Sheep given a Concentrate or Roughage Diet. Domest. Anim. Endocrinol. 10:279–87.

Adewuyi, A. A., E. Gruysi, and F. J. C. M. van Eerdenburg. 2005. Non esterified fatty acids (NEFA) in dairy cattle. A review. Vet. Q. 27:117–126. doi:10.1080/01652176.2005.9695192.

Adin, G., A. Gelman, R. Solomon, I. Flamenbaum, M. Nikbachat, E. Yosef, A. Zenou, A. Shamay, Y. Feuermann, S. J. Mabjeesh, and J. Miron. 2009. Effects of cooling dry cows under heat load conditions on mammary gland enzymatic activity, intake of food and water, and performance during the dry period and after parturition. Livest. Sci. 124:189–195. doi:10.1016/j.livsci.2009.01.014.

Ahmed, B. M. S., U. Younas, T. O. Asar, S. Dikmen, P. J. Hansen, and G. E. Dahl. 2017. Cows exposed to heat stress during fetal life exhibit improved thermal tolerance. J. Anim. Sci. 95:3497–3503. doi:10.2527/jas.2016.1298.

Akbarinejad, V., F. Gharagozlou, and M. Vojgani. 2017. Temporal effect of maternal heat stress during gestation on the fertility and anti-Müllerian hormone concentration of offspring in bovine. Theriogenology 99:69–78. doi:10.1016/j.theriogenology.2017.05.018.

Al-Dawood, A. 2017. Effect of heat stress on adipokines and some blood metabolites in goats from Jordan. Anim. Sci. J. 88:356–363. doi:10.1111/asj.12636.

Alam, M. M., M. A. Hansen, M. M. Rahman, M. M. Hossain, M. R. Haque, Z. Sobhan, and M. S. Islam. 2011. Effect of heat stress on behavior, physiological and blood parameters of goat. Progress. Agric. 22:37–45.

Ames, D. R., J. E. Nellor, and T. Adams. 1971. Energy Balance during Heat Stress in Sheep. J. Anim. Sci. 32:784–88.

Ames, D. R., and D. R. Brink. 1977. Effect of Temperature on Lamb Performance and Protein Efficiency Ratio. J. Anim. Sci. 44:136–40.

Amitha, J. P., G. Krishnan, M. Bagath, V. Sejian, and R. Bhatta. 2019. Heat Stress Impact on the Expression Patterns of Different Reproduction Related Genes in Malabari Goats. Theriogenology 131:169-176. doi:10.1016/j.theriogenology.2019.03.036.

Angel, S. P., M. Bagath, V. Sejian, G. Krishnan, and R. Bhatta. 2018. Expression patterns of candidate genes reflecting the growth performance of goats subjected to heat stress. Mol. Biol. Rep. 45:2847–2856. doi:10.1007/s11033-018-4440-0.

Angrecka, S., and P. Herbut. 2015. Conditions for cold stress development in dairy cattle kept in free stall barn during severe frosts. Czech J. Anim. Sci. 60:81–87. doi:10.17221/7978-CJAS.

AOAC International. 2003. Official Methods of Analysis. 17th ed. AOAC International, Gaithersburg, MD.

Appleman, R. D., and J. C. Delouche. 1958. Behavioral, Physiological and Biochemical Responses of Goats to Temperature, 0° to 40° C. J. Anim. Sci. 17:326–335. doi:10.2527/jas1958.172326x.

Arfuso, F., M. Rizzo, C. Giannetto, E. Giudice, F. Fazio, and G. Piccione. 2016. Age-related changes of serum mitochondrial uncoupling 1, rumen and rectal temperature in goats. J. Therm. Biol. 59:47–51. doi:10.1016/j.jtherbio.2016.05.002.

Barba-Vidal, E., V. F. B. Roll, L. Castillejos, A. A. Guerra-Ordaz, X. Manteca, J. J. Mallo, and S. M. Martín-Orúe. 2017. Response to a *Salmonella* Typhimurium challenge in piglets supplemented with protected sodium butyrate or *Bacillus licheniformis*: effects on performance, intestinal health and behavior. Transl. Anim. Sci. 1:186–200. doi:10.2527/tas2017.0021.

Barker, D., J. Eriksson, T. Forsen, and C. Osmond. 2002. Fetal Origins of Adult Disease: Strength of Effects and Biological Basis. Int. J. Epidemiol. 31:1235–39.

Barnett, M. C., J. R. Mcfarlane, and R. S. Hegarty. 2015. Low ambient temperature elevates plasma triiodothyronine concentrations while reducing digesta mean retention time and methane yield in sheep. J. Anim. Physiol. Anim. Nutr. 99:483–491. doi:10.1111/jpn.12252.

Battini, M., S. Barbieri, L. Fioni, and S. Mattiello. 2015. Feasibility and validity of animal-based indicators for on-farm welfare assessment of thermal stress in dairy goats. Int. J. Biometeorol. 60:289–296. doi:10.1007/s00484-015-1025-7.

Battini, M., A. Vieira, S. Barbieri, I. Ajuda, G. Stilwell, and S. Mattiello. 2014. Invited review: Animal-based indicators for on-farm welfare assessment for dairy goats. J. Dairy Sci. 97:6625–6648. doi:10.3168/jds.2013-7493.

De Blasio, M. J., K. L. Gatford, I. C. McMillen, J. S. Robinson, and J. A. Owens. 2007. Placental restriction of fetal growth increases insulin action, growth, and adiposity in the young lamb. Endocrinology 148:1350–1358. doi:10.1210/en.2006-0653.

Blatteis, C. M. 1998. Body Temperature. Pages 13-22 in Physiology and Pathophysiology of Temperature Regulation, C. M. Blatteis, ed. World Scientific, Singapore.

Blunden, J., and D. S. Arndt. 2019. State of the climate in 2018. Bull. Am. Meteorol. Soc. 100:Si-S305. doi:10.1175/2019BAMSStateoftheClimate.1.

Bøe, K. E., I. L. Andersen, L. Buisson, E. Simensen, and W. K. Jeksrud. 2007. Flooring preferences in dairy goats at moderate and low ambient temperature. Appl. Anim. Behav. Sci. 108:45–57. doi:10.1016/j.applanim.2006.12.002.

Bøe, K. E., and R. Ehrlenbruch. 2013. Thermoregulatory behavior of dairy goats at low temperatures and the use of outdoor yards. Can. J. Anim. Sci. 93:35–41. doi:10.4141/CJAS2012-028.

Breed M, Sanchez L. Both environment and genetic makeup influence behavior. Nature Education Knowledge. 2010; 3:68

Bristow, D. J., and D. S. Holmes. 2007. Cortisol levels and anxiety-related behaviors in cattle. Physiol. Behav. 90:626–628. doi:10.1016/j.physbeh.2006.11.015.

Brooks, K., G. Burns, and T. E. Spencer. 2014. Conceptus elongation in ruminants: roles of progesterone, prostaglandin, interferon tau and cortisol. J. Anim. Sci. Biotechnol. 5:53.

Brouček, J., M. Letkovicová, and K. Kovalcuj. 1991. Estimation of cold stress effect on dairy cows. Int. J. Biometeorol. 35:29–32.

Brown, D. L., S. R. Morrison, and G. E. Bradford. 1988. Effects of Ambient Temperature on Milk Production of Nubian and Alpine Goats. J. Dairy Sci. 71:2486–90.

Castro-Costa, A., A.A.K. Salama, X. Moll, J. Aguiló, and G. Caja. 2015. Using wireless rumen sensors for evaluating the effects of diet and ambient temperature in nonlactating dairy goats. J. Dairy Sci. 98:4646–4658. doi:10.3168/jds.2014-8819.

Chase, L.E. 2016. Cold Stress: Effects on nutritional requirements, health and performance. Ref. Module Food Sci. do:10.1016/B978-0-08-100596-5.21234-1.

Chavatte-Palmer, P., M. A. Velazquez, H. Jammes, and V. Duranthon. 2018. Review: Epigenetics, developmental programming and nutrition in herbivores. Animal 12:s363–s371. doi:10.1017/S1751731118001337.

Chen, X., A. L. Fahy, A. S. Green, M. J. Anderson, R. P. Rhoads, and S. W. Limesand. 2010. β2-Adrenergic receptor desensitization in perirenal adipose tissue in fetuses and lambs with placental insufficiency-induced intrauterine growth restriction. J. Physiol. 588:3539–3549. doi:10.1113/jphysiol.2010.192310.

Chmurzynska, A. 2010. Fetal Programming: Link between Early Nutrition, DNA Methylation, and Complex Diseases. Nutr. Rev. 68:87–98.

Cleal, J. K., K. R. Poore, J. P. Boullin, O. Khan, R. Chau, O. Hambidge, C. Torrens, J. P. Newman, L. Poston, D. E. Noakes, M. A. Hanson, and L. R. Green. 2007. Mismatched pre- and postnatal nutrition leads to cardiovascular dysfunction and altered renal function in adulthood. Proc. Natl. Acad. Sci. U. S. A. 104:9529–9533. doi:10.1073/pnas.0610373104.

Constable, P. D., K. W. Hinchcliff, S. H. Done, and W. Gruenberg. 2016. General systemic states. 11th ed. Saunders Ltd, St Louis, Missouri.

Constantinou, A. 1987. Goat housing for different environments and production systems. Pages 241–268 in Proceedings Fourth International Conference on Goats, Brasilia, Brazil.

Contreras-Jodar, A., N. H. Nayan, S. Hamzaoui, G. Caja, and A. A. K. Salama. 2018. Heat Stress Modifies the Lactational Performances and the Urinary Metabolomic Profile Related to Gastrointestinal Microbiota of Dairy Goats. PLoS One 14:e0202457.

Coulon, M., F. Lévy, C. Ravel, R. Nowak, and A. Boissy. 2014. Mild effects of gestational stress and social reactivity on the onset of mother – young interactions and bonding in sheep. Int. J. Biol. Stress 17:460–470. doi:10.3109/10253890.2014.969238.

Coulon, M., R. Nowak, S. Andanson, B. Petit, F. Lévy, and A. Boissy. 2015. Effects of prenatal stress and emotional reactivity of the mother on emotional and cognitive abilities in lambs. Dev. Psychobiol. 57:626–636. doi:10.1002/dev.21320.

Cowie, A. T. 1971. Influence of hormones on mammary growth and milk secretion. I.R. Falconer, ed. Butterworths, London, UK.

Curtis, S. E. 1981. Environmental management in animal agriculture. 2nd ed. Mahomet, IL.

Dahl, G.E., S. Tao, and J. Laporta. 2017. Triennial lactation symposium/bolfa: Late gestation heat stress of dairy cattle programs dam and daughter milk production. J. Anim. Sci. 95:5701–5710. doi:10.2527/jas2017.2006.

Dangi, S. S., M. Gupta, V. Nagar, V. P. Yadav, S. K. Dangi, O. Shankar, V. S. Chouhan, P. Kumar, G. Singh, and M. Sarkar. 2014. Impact of short-term heat stress on physiological responses and expression profile of HSPs in Barbari goats. Int. J. Biometeorol. 58:2085–2093. doi:10.1007/s00484-014-0809-5.

Dangi, S. S., S. K. Dangi, V. S. Chouhan, M. R. Verma, P. Kumar, G. Singh, and M. Sarkar. 2016. Modulatory effect of betaine on expression dynamics of HSPs during heat stress acclimation in goat (*Capra hircus*). Gene 575:543–550. doi:10.1016/j.gene.2015.09.031.

Dodic, M., A. Peers, K. Moritz, V. Hantzis, and E. M. Wintour. 2002. No evidence for HPA reset in adult sheep with high blood pressure due to short prenatal exposure to dexamethasone. Am. J. Physiol. Regul. Integr. Comp. Physiol. 282:343–350. doi:10.1152/ajpregu.00222.2001.

Du, M., J. Tong, J. Zhao, K. R. Underwood, M. Zhu, S. P. Ford, and P. W. Nathanielsz. 2010. Fetal programming of skeletal muscle development in ruminant animals. J. Anim. Sci. 88:E51–E60. doi:10.2527/jas.2009-2311.

Dutt, R. H. 1963. Critical period for early embryo mortality in ewes exposed to high temperature. J. Anim. Sci. 22:713–719. doi:10.2527/jas1963.223713x.

Ekpe, E. D., and R. J. Christopherson. 2000. Metabolic and endocrine responses to cold and feed restriction in ruminants. Can. J. Anim. Sci. 80:87–95. doi:10.4141/A99-028.

El-Zarei, M. F., A. M. Alseaf, A. A. Alhaidary, E. F. Mousa, A. B. Okab, E. M. Samara, and K. A. Abdoun. 2019. Short-term heat shock proteins 70 and 90 mRNA expression profile and its relation to thermo-physiological parameters in goats exposed to heat stress. Int. J. Biometeorol. 63:459–465. doi:10.1007/s00484-019-01677-2.

Estanislau, C., and S. Morato. 2005. Prenatal stress produces more behavioral alterations than maternal separation in the elevated plus-maze and in the elevated T-maze. Behav. Brain Res. 163:70–77. doi:10.1016/j.bbr.2005.04.003.

Everitt, G. C. 1964. Maternal undernutrition and retarded foetal development in Merino sheep. Nature 201:1341–1342.

Fabris, T. F., J. Laporta, A. L. Skibiel, F. N. Corra, B. D. Senn, S. E. Wohlgemuth, and G. E. Dahl. 2019. Effect of heat stress during early, late, and entire dry period on dairy cattle. J. Dairy Sci. 102:5647–5656. doi:10.3168/jds.2018-15721.

Faulkner, A., E. M. Thomson, J. M. Bassett, and G. E. Thomson. 1980. Cold exposure and mammary glucose metabolism in the lactating goat. Br. J. Nutr. 43:163–170.

Faurie, A. S., D. Mitchell, and H. P. Laburn. 2001. Feto-maternal relationships in goats during heat and cold exposure. Physiol. Soc. 86:199–204.

Finch, V. A. 1986. Body Temperature in Beef Cattle: Its Control and Relevance to Production in the Tropics. J. Anim. Sci. 62:531–42.

Forbes, J. M. 1967. Factors affecting the gestation length in sheep. J. Agric. Sci. 68:191. doi:10.1017/S0021859600016221.

Ford, S. P., B. W. Hess, M.M. Schwope, M. J. Nijland, J. S. Gilbert, K. A. Vonnahme, W. J. Means, H. Han, and P. W. Nathanielsz. 2007. Maternal undernutrition during early to mid-gestation in the ewe results in altered growth, adiposity, and glucose tolerance in male offspring. J. Anim. Sci. 85:1285–1294. doi:10.2527/jas.2005-624.

Fox, D. G., and T. P. Tylutki. 1998. Accounting for the Effects of Environment on the Nutrient Requirements of Dairy Cattle. J. Dairy Sci. 81:3085–95.

Freer, M., H. Dove, and J. V. Nolan. 2007. Nutrient Requirements of Domesticated Ruminants. CSIRO Publishing, Collingwood, Australia.

Funston, R. N., D. M. Larson, and K. A. Vonnahme. 2010. Effects of maternal nutrition on conceptus growth and offspring performance : Implications for beef cattle production. J. Anim. Sci. 88:E205–E215. doi:10.2527/jas.2009-2351.

Funston, R. N., and A. F. Summers. 2013. Epigenetics: Setting up lifetime production of beef cows by managing nutrition. Ann. Rev. Anim. Biosci. 1:339–363. doi:10.1146/annurev-animal-031412-103649.

Galán, E., P. Llonch, A. Villagrá, H. Levit, S. Pinto, and A. del Prado. 2018. A systematic review of non-productivityrelated animal-based indicators of heat stress resilience in dairy cattle. PLoS One 13:1–19. doi:10.1371/journal.pone.0206520.

Gao, S. T., J. Guo, S. Y. Quan, X. M. Nan, M. V. Sanz Fernandez, L. H. Baumgard, and D. P. Bu. 2017. The effects of heat stress on protein metabolism in lactating Holstein cows. J. Dairy Sci. 100:5040–5049. doi:10.3168/jds.2016-11913.

Gaughan, J. B., and T. L. Mader. 2009. Effects of sodium chloride and fat supplementation on finishing steers exposed to hot and cold conditions. J. Anim. Sci. 87:612–621. doi:10.2527/jas.2008-1125.

Gonzalez-Rivas, P. A., K. DiGiacomo, P. A. Giraldo, B. J. Leury, J. J. Cottrell, and F. R. Dunshea. 2017. Reducing Rumen Starch Fermentation of Wheat with Three Percent Sodium Hydroxide Has the Potential to Ameliorate the Effect of Heat Stress in Grain-Fed Wethers. J. Anim. Sci. 95:5547–62.

Graham, A. D., R. J. Christopherson, and J. R. Thompson. 1981. Endocrine and metabolic changes in sheep associated with acclimatation to constant intermittent cold exposure. Can. J. Anim. Sci. 61:81–90. doi:10.4141/cjas81-012.

Grigsby, P. L. 2016. Animal models to study placental development and function throughout normal and dysfunctional human pregnancy. Semin. Reprod. Med. 34:11–16. doi:10.1055/s-0035-1570031.

Guilloteau, P., R. Zabielski, and J. W. Blum. 2009. Gastrointestinal Tract and Digestion in the Young Ruminant: Ontogenesis, Adaptations, Consequences and Manipulations. J. Physiol. Pharmacol. 60:37–46.

Hall, G. M., J. N. Lucke, R. Lovell, and D. Lister. 1980. Porcine Malignant Hyperthermia. VII: Hepatic Metabolism. Br. J. Anaesth. 52:11–17.

Hamzaoui, S., A. A. K. Salama, E. Albanell, X. Such, and G. Caja. 2013. Physiological responses and lactational performances of late-lactation dairy goats under heat stress conditions. J. Dairy Sci. 96:6355–6365. doi:10.3168/jds.2013-6665.

Hansen, P. J. 2004. Physiological and Cellular Adaptations of Zebu Cattle to Thermal Stress.

Anim. Reprod. Sci. 82–83:349–60.

Hansen, P. J. 2015. Developmental programming in the preimplantation period: can it be exploited to enhance postnatal function in cattle?. Anim. Reprod. 12:428–436.

Haque, N., A. Ludri, S. A. Hossain, and M. Ashuntosh. 2012a. Alteration of metabolic profiles in young and adult Murrah buffaloes exposed to acute heat stress. Int. J. Appl. Anim. Sci. 1:23–29.

Haque, N., A. Ludri, S. A. Hossain, and M. Ashutosh. 2012b. Comparative studies on temperature threshold for heat shock protein 70 induction in young and adult Murrah buffaloes: Comparative studies on temperature threshold for heat shock protein 70 induction in young and adult Murrah buffaloes. J. Anim. Physiol. Anim. Nutr. 96:920–929. doi:10.1111/j.1439-0396.2011.01208.x.

Hart, I. C. 1973. Effect of 2-bromo-α-ergocryptine on milk yield and the level of prolactin and growth hormone in the blood of the goat at milking. J. Endocrinol. 57:179–180. doi:10.1677/joe.0.0570179.

Hayirli, A., D. R. Bremmer, S. J. Bertics, M. T. Socha, and R. R. Grummer. 2001. Effect of Chromium Supplementation on Production and Metabolic Parameters in Periparturient Dairy Cows. J. Dairy Sci. 84:1218–30.

Hayirli, A. 2006. The Role of Exogenous Insulin in the Complex of Hepatic Lipidosis and Ketosis Associated with Insulin Resistance Phenomenon in Postpartum Dairy Cattle. Vet. Res. Com. 30:749–74.

Herbut, P., S. Angrecka, and J. Walczak. 2018. Environmental parameters to assessing of heat stress in dairy cattle—a review. Int. J. Biometeorol. 62:2089–2097. doi:10.1007/s00484-018-1629-9.

Herskin, M. S., L. Munksgaard, and J. Ladewig. 2004. Effects of acute stressors on nociception, adrenocortical responses and behavior of dairy cows. Physiol. Behav. 83:411–420. doi:10.1016/j.physbeh.2004.08.027.

Hirayama, T., and K. Katoh. 2004. Effects of Heat Exposure and Restricted Feeding on Behavior, Digestibility and Growth Hormone Secretion in Goats. Asian-Australas. J. Anim. Sci. 17:655–58.

Holtenius, P., and K. Holtenius. 1996. New aspects of ketone bodies in energy metabolism of dairy cows: A review. J. Vet. Med. Ser. A Physiol. Pathol. Clin. Med. 43:579–587.

Hooper, H. B., P. dos Santos Silva, S. Aparecida de Oliveira, G. K. Fonseca Merighe, and J. A. Negrão. 2018. Acute heat stress induces changes in physiological and cellular responses in Saanen goats. Int. J. Biometeorol. 62:2257–2265. doi:https://doi.org/10.1007/s00484-018-1630-3.

Van den Hove, D. L. A., N.K. Leibold, E. Strackx, M. Martinez-Claros, K. P. Lesch, H. W. M. Steinbusch, K. R. J. Schruers, and J. Prickaerts. 2014. Prenatal stress and subsequent exposure to chronic mild stress in rats; interdependent effects on emotional behavior and the serotonergic system. Eur. Neuropsychopharmacol. 24:595–607. doi:10.1016/j.euroneuro.2013.09.006.

INRA. 2018. INRA Feeding System for Ruminants. Ed. Wageningen Academic, Wageningen, The Netherlands.

Itoh, F., Y. Obara, H. Fuse, M.T. Rose, I. Osaka, and H. Takahashi. 1997. Effects of cold exposure on responses of plasma insulin, glucagon, and metabolites in heifers. J. Anim. Physiol. a. Anim. Nutr. 78:31-41.

Jaquiery, A. L., M. H. Oliver, M. Honeyfield-Ross, J. E. Harding, and F. H. Bloomfield. 2012. Periconceptional undernutrition in sheep affects adult phenotype only in males. J. Nutr. Metab. 123610. doi:10.1155/2012/123610.

Jeelani, R., D. Konwar, A. Khan, D. Kumar, D. Chakraborty, and B. Brahma. 2019. Reassessment of temperature-humidity index for measuring heat stress in crossbred dairy cattle of a sub-tropical region. J. Therm. Biol. 82:99–106. doi:10.1016/j.jtherbio.2019.03.017.

Johnson, H. D. 1976. Progress in Animal Biometerology. 1st ed. H.D. Johnson, ed. Swets & Zeitling, Amsterdam, The Netherlands.

Johnson, J. S., M. V. Sanz Fernandez, N. A. Gutierrez, J. F. Patience, J. W. Ross, N. K. Gabler, M. C. Lucy, T. J. Safranski, R. P. Rhoads, and L. H. Baumgard. 2015a. Effects of in Utero Heat Stress on Postnatal Body Composition in Pigs: I. Growing Phase. J. Anim. Sci. 93:71–81.

Johnson, J. S., M. V. Sanz Fernandez, J. T. Seibert, J. W. Ross, M. C. Lucy, T. J. Safranski, T. H. Elsasser, S. Kahl, R. P. Rhoads, and L. H. Baumgard. 2015b. In Utero Heat Stress Increases Postnatal Core Body Temperature in Pigs. J. Anim. Sci. 93:4312–22.

Kadzere, C. T., M. R. Murphy, N. Silanikove, and E. Maltz. 2002. Heat stress in lactating dairy cows: A review. Livest. Prod. Sci. 77:59–91. doi:10.1016/S0301-6226(01)00330-X.

Kakumani, R., O. Ahmad, and V. Devabhaktuni. 2012. Identification of CpG islands in DNA sequences using statistically optimal null filters. EURASIP J. Bioinform. Syst. Biol. 12:1–14.

Kaliber, M., N. Koluman, and N. Silanikove. 2016. Physiological and behavioral basis for the successful adaptation of goats to severe water restriction under hot environmental conditions. Animal 10:82–88. doi:10.1017/S1751731115001652.

Kang, H. J., I. K. Lee, M. Y. Piao, M. J. Gu, C. H. Yun, H. J. Kim, and M. Baik. 2016. Effects of ambient temperature on growth performance, blood metabolites and immune cell populations in Korean cattle steers. Asian-Australas. J. Anim. Sci. 29:436–443. doi:10.5713/ajas.15.0937.

Kennedy, P. M. 1985. Influences of cold exposure on digestion of organic matter, rates of passage of digesta in the gastrointestinal tract, and feeding and rumination behaviour in sheep given four forage diets in the chopped, or ground and pelleted form. Br. J. Nutr. 53:159–73. doi:10.1079/BJN19850020.

Khounsy, S., S. Nampanya, P. Inthavong, M. Yang, B. Khamboungheung, M. Avery, R. Bush, L. Rast, and P. A. Windsor. 2012. Significant mortality of large ruminants due to hypothermia in northern and central Lao PDR. Trop. Anim. Health Prod. 44:835–842. doi:10.1007/s11250-011-9975-1.

Knittle, J. L., and J. Hirsch. 1968. Effect of Early Nutrition on the Development of Rat Epididymal

Fat Pads: Cellularity and Metabolism. J. Clin. Invest. 47:2091–98.

Kour, G., N. Kataria, and N. S. Lawhale. 2014. Ambient stress associated variations in yotal serum proteins and total fatty acids in Marwari goats. Glob. Vet. 13:337–341. doi:10.5829/idosi.gv.2014.13.03.8519.

Kour, S., J. Devi, K. Kour, D. Chakraborty, A. W. Ganai, R. Zargar, and P. Khajuria. 2017. Effect of extreme seasons on some blood biochemical parameters in Beetal and Toggenberg goats of Jammu region. J. Anim. Res. 7:779–784. doi:10.5958/2277-940X.2017.00119.X.

Kovács, L., F. L. Kézér, M. Bakony, V. Jurkovich, and O. Szenci. 2018. Lying down frequency as a discomfort index in heat stressed Holstein bull calves. Sci. Rep. 8:15065. doi:10.1038/s41598-018-33451-6.

Lambertz, C., C. Sanker, and M. Gauly. 2014. Climatic effects on milk production traits and somatic cell score in lactating Holstein-Friesian cows in different housing systems. J. Dairy Sci. 97:319–329. doi:10.3168/jds.2013-7217.

Laporta, J., T. F. Fabris, A. L. Skibiel, J. L. Powell, M. J. Hayen, K. Horvath, E. K. Miller-Cushon, and G.E. Dahl. 2017. In utero exposure to heat stress during late gestation has prolonged effects on the activity patterns and growth of dairy calves. J. Dairy Sci. 100:2976–2984. doi:10.3168/jds.2016-11993.

Lenis Sanin, Y., A. M. Zuluaga Cabrera, and A. M. Tarazona Morales. 2016. Adaptive responses to thermal stress in mammals. Rev. Médica Vet. 31:121–135.

Li, B. T., R. J. Christopherson, and S. J. Cosgrove. 2000. Effect of water restriction and environmental temperatures on metabolic rate and physiological parameters in sheep. Can. J. Anim. Sci. 80:97–104. doi:10.4141/A99-041.

Li, F. K., Y. Yang, K. Jenna, C. H. Xia, S. J. Lv, and W. H. Wei. 2018. Effect of heat stress on the behavioral and physiological patterns of Small-tail Han sheep housed indoors. Trop. Anim. Health Prod. 50:1893–1901. doi:10.1007/s11250-018-1642-3.

Limesand, S. W., P. J. Rozance, D. Smith, and W. W. Hay. 2007. Increased insulin sensitivity and maintenance of glucose utilization rates in fetal sheep with placental insufficiency and intrauterine growth restriction. Am. J. Physiol. Metab. 293:1716–1725. doi:10.1152/ajpendo.00459.2007.

Long, N. M., M. J. Prado-Cooper, C. R. Krehbiel, U. Desilva, and R. P. Wettemann. 2010. Effects of nutrient restriction of bovine dams during early gestation on postnatal growth, carcass and organ characteristics, and gene expression in adipose tissue and muscle. J. Anim. Sci. 88:3251–3261. doi:10.2527/jas.2009-2512.

Lopes, L. B., C. Eckstein, D. S. Pina, and R. A. Carnevalli. 2016. The influence of trees on the thermal environment and behaviour of grazing heifers in Brazilian Midwest. Trop. Anim. Health Prod. 48:755–761. doi:10.1007/s11250-016-1021-x.

Louey, S., M. L. Cock, and R. Harding. 2005. Long Term Consequences of Low Birthweight on Postnatal Growth, Adiposity and Brain Weight at Maturity in Sheep. J. Reprod. Develop. 51:59–68.

Mabjeesh, S. J., C. Sabastian, O. Gal-Garber, and A. Shamay. 2013. Effect of photoperiod in the third trimester of gestation on milk production and circulating hormones in dairy goats. J. Dairy Sci. 96:699–705. doi:10.3168/jds.S0022-0302(07)71553-9.

Maloiy, G. M. O., T. I. Kanui, P. K. Towett, S. N. Wambugu, J. O. Miaron, and M. M. Wanyoike. 2008. Effects of dehydration and heat stress on food intake and dry matter digestibility in East African ruminants. Comp. Biochem. Physiol. - A Mol. Integr. Physiol. 151:185–190. doi:10.1016/j.cbpa.2008.06.019.

Marchesini, G., M. Cortese, D. Mottaran, R. Ricci, L. Serva, B. Contiero, S. Segato, and I. Andrighetto. 2018. Effects of axial and ceiling fans on environmental conditions, performance and rumination in beef cattle during the early fattening period. Livest. Sci. 214:225–230. doi:10.1016/j.livsci.2018.06.009.

Marchlewska-Koj, A., J. Kapusta, and M. S. Kruczek. 2003. Prenatal stress modifies behavior in offspring of bank voles (*Clethrionomys glareolus*). Physiol. Behav. 79:671–678. doi:10.1016/S0031-9384(03)00168-9.

Martin, P., and P. Bateson. 2007. Recording methods. 3rd ed. P. Martin and P. Bateson, ed. Cambridge University Press, Cambridge.

Martin, K. M., P. McGilchrist, J. M. Thompson, and G. E. Gardner. 2011. Progeny of High Muscling Sires Have Reduced Muscle Response to Adrenaline in Sheep. Animal. 5:1060–70.

Matthews, S. G., D. Owen, S. Banjanin, and M. H. Andrews. 2002. Glucocorticoids, hypothalamo-pituitary-adrenal (HPA) development, and life after birth. Endocr. Res. 28:709–718. doi:10.1081/ERC-120016991.

McBride, G. E., and R. J. Christopherson. 1984. Effects of cold exposure on young growing lambs. Can. J. Anim. Sci. 64:403–410. doi:10.4141/cjas84-046.

McGeady, T. A., P. J. Quinn, E. S. Fitzpatrick, M. T. Ryan, D. Kilroy, and P. Lonergan. 2017. Veterinary Embryology. 2nd ed. Wiley-Blackwell.

McGowan, P. O., and S. G. Matthews. 2018. Prenatal stress, glucocorticoids, and developmental programming of the stress response. Endocrinology 159:69–82. doi:10.1210/en.2017-00896.

McGregor, B.A. 2002. Extent and source of short and cotted mohair. Barton, Australia.

Mehaba, N., A. A. K. Salama, X. Such, E. Albanell, and G. Caja. 2019. Lactational Responses of Heat-Stressed Dairy Goats to Dietary L-Carnitine Supplementation. Animals 9:567.

Min, L., J. Cheng, B. Shi, H. Yang, N. Zheng, and J. Wang. 2015. Effects of heat stress on serum insulin, adipokines, AMP-activated protein kinase, and heat shock signal molecules in dairy cows. J. Zhejiang Univ. B 16:541–548. doi:10.1631/jzus.b1400341.

Min, L., S. Zhao, H. Tian, X. Zhou, Y. Zhang, S. Li, H. Yang, N. Zheng, and J. Wang. 2017. Metabolic responses and "omics" technologies for elucidating the effects of heat stress in dairy cows. Int. J. Biometeorol. 61:1149–1158. doi:10.1007/s00484-016-1283-z.

Monteiro, A. P. A., S. Tao, I. M. Thompson, and G. E. Dahl. 2014. Effect of heat stress during late gestation on immune function and growth performance of calves: Isolation of altered colostral and calf factors. J. Dairy Sci. 97:6426–6439. doi:10.3168/jds.2013-7891.

Monteiro, A. P. A., J. R. Guo, X. S. Weng, B. M. Ahmed, M. J. Hayen, G. E. Dahl, J. K. Bernard, and S. Tao. 2016a. Effect of maternal heat stress during the dry period on growth and metabolism of calves. J. Dairy Sci. 99:3896–3907. doi:10.3168/jds.2015-10699.

Monteiro, A. P. A., S. Tao, I. M. T. Thompson, and G. E. Dahl. 2016b. In Utero Heat Stress Decreases Calf Survival and Performance through the First Lactation. J. Dairy Sci. 99:8443–50.

Morera, P., L. Basiricò, K. Hosoda, and U. Bernabucci. 2012. Chronic heat stress up-regulates leptin and adiponectin secretion and expression and improves leptin, adiponectin and insulin sensitivity in mice. J. Mol. Endocrinol. 48:129–138. doi:10.1530/jme-11-0054.

Morrison, J. L. 2008. Sheep models of intrauterine growth restriction: fetal adaptations and consequences. Clin. Exp. Pharmacol. Physiol. 35:730–743. doi:10.1111/j.1440-1681.2008.04975.x.

Mourshed, M. 2016. Climatic parameters for building energy applications: A temporal-geospatial assessment of temperature indicators. Renew. Energy 94:55–71. doi:10.1016/j.renene.2016.03.021.

NRC. 1971. A Guide to Environmental Research on Animals. National Academies Press, Washington, DC.

Nuyt, A. M., and M. Szyf. 2007. Developmental programming through epigenetic changes. Circ. Res. 100:452–455. doi:10.1161/01.RES.0000260292.95612.ac.

O'Brien, M. D., R. P. Rhoads, S. R. Sanders, G. C. Duff, and L. H. Baumgard. 2010. Metabolic Adaptations to Heat Stress in Growing Cattle. Domest. Anim. Endocrinol. 38:86–94.

Palma-Gudiel, H., A. Córdova-Palomera, E. Eixarch, M. Deuschle, and L. Fañanás. 2015. Maternal psychosocial stress during pregnancy alters the epigenetic signature of the glucocorticoid receptor gene promoter in their offspring: a meta-analysis. Epigenetics 10:893–902. doi:10.1080/15592294.2015.1088630.

Pelham, H. R. B. 1984. Hsp7O accelerates the after heat shock. EMBO J. 3:3095–3100.

Pohlin, F., K. Brabender, G. Fluch, G. Stalder, T. Petit, and C. Walzer. 2017. Seasonal variations in heart rate variability as an indicator of stress in free-ranging pregnant Przewalski's horses (*E. ferus* przewalskii) within the Hortobágy National Park in Hungary. Front. Physiol. 8:1–11. doi:10.3389/fphys.2017.00664.

Pragna, P., V. Sejian, M. Bagath, G. Krishnan, P. R. Archana, N. M. Soren, V. Beena, and R. Bhatta. 2018. Comparative assessment of growth performance of three different indigenous goat breeds exposed to summer heat stress. J. Anim. Physiol. Anim. Nutr. 102:825–836. doi:10.1111/jpn.12892.

Rae, M. T., C. E. Kyle, D. W. Miller, A. J. Hammond, A. N. Brooks, and S. M. Rhind. 2002. The effects of undernutrition, in utero, on reproductive function in adult male and female sheep. Anim. Reprod. Sci. 72:63–71.

Ramón, M., C. Díaz, M. D. Pérez-Guzman, and M. J. Carabaño. 2016. Effect of exposure to adverse climatic conditions on production in Manchega dairy sheep. J. Dairy Sci. 99:5764–5779. doi:10.3168/jds.2016-10909.

Resende, K. T., M. H. Machado da Rocha Fernandes, F. Hentz, I. A. Molina de Almeida Teixeira, and J. A. Garcia. 2017. Métodos de Estimativa Da Composição Corporal de Caprinos Em Crescimento. Acta Sci. Anim. Sci. 39:189–94.

Rhoads, M. L., R. P. Rhoads, M. J. VanBaale, R. J. Collier, S. R. Sanders, W. J. Weber, B. A. Crooker, and L. H. Baumgard. 2009. Effects of Heat Stress and Plane of Nutrition on Lactating Holstein Cows: I. Production, Metabolism, and Aspects of Circulating Somatotropin. J. Dairy Sci. 92:1986–1997. doi:10.3168/jds.2008-1641

Romeyer, A., and M. F. Bouissou. 1992. Assessment of fear reactions in domestic sheep, and influence of breed and rearing conditions. Appl. Anim. Behav. Sci. 34:93–119.

Roseboom, T., S. de Rooij, and R. Painter. 2006. The Dutch Famine and Its Long-Term Consequences for Adult Health. Early Hum. Dev. 82:485–91.

Roussel, S., A. Boissy, D. Montigny, P. H. Hemsworth, and C. Duvaux-Ponter. 2005. Gender-specific effects of prenatal stress on emotional reactivity and stress physiology of goat kids. Horm. Behav. 47:256–266. doi:10.1016/j.yhbeh.2004.09.010.

Ruiz-Miranda, C. R., and M. Callard. 1992. Effects of the presence of the mother on responses of domestic goat kids (*Capra hircus*) to novel inanimate objects and humans. Appl. Anim. Behav. Sci. 33:277–285. doi:10.1016/S0168-1591(05)80015-2.

Salama, A. A. K., G. Caja, S. Hamzaoui, B. Badaoui, A. Castro-Costa, M. M. Guilhermino, and R. Bozzi. 2014. Different levels of response to heat stress in dairy goats. Small Rumin. Res. 121:73–79. doi:10.1016/j.smallrumres.2013.11.021.

Salama, A. A. K., G. Caja, S. Hamzaoui, X. Such, E. Albanell, B. Badaoui, and J. J. Loor. 2016. Thermal stress in ruminants: Responses and strategies for alleviation. J. J. Villalba and X. Manteca, ed. 5m Publishing, Sheffield, UK.

Samara, E. M., K. A. Abdoun, A. B. Okab, M. A. Al-Badwi, M. F. El-Zarei, A. M. Al-Seaf, and A. A. Al-Haidary. 2016. Assessment of heat tolerance and production performance of Aardi, Damascus, and their crossbred goats. Int. J. Biometeorol. 60:1377–1387. doi:10.1007/s00484-015-1131-6.

Sano, H., K. Ambo, and T. Tsuda. 1985. Blood Glucose Kinetics in Whole Body and Mammary Gland of Lactating Goats Exposed to Heat. J. Dairy Sci. 68:2557–64.

Sano, H., S. Matsunobu, T. Abe, and Y. Terashima. 1992. Combined effects of diet and cold exposure on insulin responsiveness to glucose and tissue responsiveness to insulin in sheep. J. Anim. Sci. 3514–3520. doi:10.1046/j.1439-0396.2001.00345.x.

Sano, H., T. Fujita, M. Murakami, and A. Shiga. 1996. Stimulative effect of epinephrine on glucose production and utilization rates in sheep using a stable isotope. Domest. Anim. Endocrinol. 13:445–451.

Sano, H., A. Takebayashi, Y. Kodama, K. Nakamura, H. Ito, Y. Arino, T. Fujita, H. Takahashi, and K. Ambo. 1999. Effects of feed restriction and cold exposure on glucose metabolism in response to feeding and insulin in sheep. J. Anim. Sci. 77:2564–2573. doi:10.2527/1999.7792564x.

Sano, H., H. Sawada, A. Takenami, S. Oda, and M. Al-Mamun. 2007. Effects of dietary energy intake and cold exposure on kinetics of plasma glucose metabolism in sheep. J. Anim. Physiol. Anim. Nutr. 91:1–5. doi:10.1111/j.1439-0396.2006.00634.x.

Sanz Sampelayo, M. R., I. Prieto, M. J. Lupiani, F. Gil Extremera, and J. Boza. 2000. Physiological responses of pre-ruminant kid goats and lambs to different environmental temperatures. J. Physiol. Biochem. 56:223–230.

Sasaki, Y., and H. Takahashi. 1980. Insulin secretion in sheep exposed to cold. J. Physiol. 306:323–35. doi:10.1113/jphysiol.1980.sp013399.

Sasaki, Y., and T. E. C. Weekes. 1986. Metabolic response to cold. L.P. Milligan, W.L. Grovum, and A. Dobson, ed. Reston Publishers Co., Reston, VA.

Schneider, M. L., C. F. Moore, G. W. Kraemer, A. D. Roberts, and O. T. DeJesus. 2002. The impact of prenatal stress, fetal alcohol exposure, or both on development: perspectives from a primate model. Psychoneuroendocrinology 27:285–298. doi:10.1016/S0306-4530(01)00050-6.

Schroeder, M., T. Sultany, and A. Weller. 2012. Prenatal stress effects on emotion regulation differ by genotype and sex in prepubertal rats. Dev. Comp. Immunol. 55:176–192. doi:10.1002/dev.21010.

Seckl, J. R. 2001. Glucocorticoid programming of the fetus; adult phenotypes and molecular mechanisms. Mol. Cell. Endocrinol. 185:61–71. doi:10.1016/S0303-7207(01)00633-5.

Sejian, V., and R. S. Srivastava. 2010. Effects of melatonin on adrenal cortical functions of Indian goats under thermal stress. Vet. Med. Int. 2010:348919. doi:10.4061/2010/348919.

Settivari, R. S., J. N. Spain, M. R. Ellersieck, J. C. Byatt, R. J. Collier, and D. E. Spiers. 2007. Relationship of Thermal Status to Productivity in Heat-Stressed Dairy Cows Given Recombinant Bovine Somatotropin. J. Dairy Sci. 90:1265–80.

Shilja, S., V. Sejian, M. Bagath, A. Mech, C. G. David, E. K. Kurien, G. Varma, and R. Bhatta. 2016. Adaptive capability as indicated by behavioral and physiological responses, plasma HSP70 level, and PBMC HSP70 mRNA expression in Osmanabadi goats subjected to combined (heat and nutritional) stressors. Int. J. Biometeorol. 60:1311–1323. doi:10.1007/s00484-015-1124-5.

Shrikhande, G. B., A. M. Rode, M. S. Pradhan, and A. K. Satpute. 2008. Seasonal effect on the composition of blood in cattle. Vet. World 1:341–342. doi:10.5455/vetworld.2008.341-342.

Silanikove, N. 2000. The physiological basis of adaptation in goats to harsh environments. Small Rumin. Res. 35:181–193. doi:10.1016/S0921-4488(99)00096-6.

Silanikove, N., and N. K. Darcan. 2015. Impact of climate change on the dairy industry in temperate zones: Predications on the overall negative impact and on the positive role of dairy goats in adaptation to earth warming. Small Rumin. Res. 23:27–37. doi:10.1016/j.smallrumres.2014.11.005.

Sinclair, K. D., C. Allegrucci, R. Singh, D. S. Gardner, S. Sebastian, J. Bispham, A. Thurston, J. F. Huntley, W. D. Rees, C. A. Maloney, R. G. Lea, J. Craigon, T. G. Mcevoy, and L. E. Young. 2007. DNA methylation, insulin resistance, and blood pressure in offspring determined by maternal periconceptional B vitamin and methionine status. PNAS 104:19351–19356. doi:10.1073/pnas.0707258104.

Singh, K., A. J. Molenaar, K. M. Swanson, B. Gudex, J. A. Arias, R. A. Erdman, and K. Stelwagen. 2012. Epigenetics: a possible role in acute and transgenerational regulation of dairy cow milk production. Animal 6:375–381. doi:10.1017/S1751731111002564.

Sivakumar, A. V. N., G. Singh, and V. P. Varshney. 2010. Antioxidants supplementation on acid base balance during heat stress in goats. Asian-Australas. J. Anim. Sci. 23:1462–68.

Skibiel, A. L., F. Peñagaricano, R. Amorín, B. M. Ahmed, G. E. Dahl, and J. Laporta. 2018. In utero heat stress alters the offspring epigenome. Sci. Rep. 8:14609. doi:10.1038/s41598-018-32975-1.

St-Pierre, N. R., B. Cobanov, and G. Schnitkey. 2003. Economic losses from heat stress by U.S. livestock industries. J. Dairy Sci. 86:E52–E77.

Stevens, A., G. Begum, A. Cook, K. Connor, C. Rumball, M. Oliver, J. Challis, F. Bloomfield, and A. White. 2010. Epigenetic changes in the hypothalamic proopiomelanocortin and glucocorticoid receptor genes in the ovine fetus after periconceptional undernutrition. Endocrinology 151:3652–3664. doi:10.1210/en.2010-0094.

Storbeck, K. H., A. C. Swart, and P. Swart. 2009. CYP17 causes hypocortisolism in the South African Angora goat. Mol. Cell. Endocrinol. 300:121–125. doi:10.1016/j.mce.2008.09.009.

Tao, S., A. P. A. Monteiro, I.M. Thompson, M. J. Hayen, and G. E. Dahl. 2012. Effect of late-gestation maternal heat stress on growth and immune function of dairy calves. J. Dairy Sci. 95:7128–7136. doi:10.3168/jds.2012-5697.

Tao, S., and G. E. Dahl. 2013. Invited review: Heat stress effects during late gestation on dry cows and their calves. J. Dairy Sci. 96:4079–4093. doi:10.3168/jds.2012-6278.

Tao, S., A. P. A. Monteiro, M. J. Hayen, and G. E. Dahl. 2014. Short communication: Maternal heat stress during the dry period alters postnatal whole-body insulin response of calves. J. Dairy Sci. 97:897–901. doi:10.3168/jds.2013-7323.

Tarr, B. 2010. Cold Stress in Cows. Accessed May 6, 2019. http://www.omafra.gov.on.ca/english/livestock/beef/facts/07-001.htm.

Thom, E. C. 1959. The discomfort index. Weatherwise 12:57–61. doi:10.1080/00431672.1959.9926960.

Thompson, G. E., and E. M. Thomson. 1972. Effect of cold exposure on mammary circulation, oxygen consumption and milk secretion in the goat. J. Physiol. 272:187–196. doi:https://doi.org/10.1113/jphysiol.1977.sp012040.

Thompson, G. E., W. Manson, P. L. Clarke, and A. W. Bell. 1978. Acute cold exposure and the metabolism of glucose and some of its precursors in the liver of the fed and fasted sheep. Q. J. Exp. Physiol. Cogn. Med. Sci. 63:189-199.

Thomson, E. M., A. M. Snoswell, P. L. Clarke, and G. E. Thompson. 1979. Effect of old exposure on mammary gland uptake of fat precursors and secretion of milk fat and carnitine in the goat. Q. J. Exp. Physiol. Cogn. Med. Sci. 64:7–15. doi:10.1113/expphysiol.1979.sp002460.

Torlińska, T., R. Banach, J. Paluszak, and A. Gryczka-Dziadecka. 1987. Hyperthermia Effect on Lipolytic Processes in Rat Blood and Adipose Tissue. Acta Physiol. Pol. 38:361–66.

Tucker, H. A., and R. P. Wettemann. 1976. Effects of Ambient Temperature and Relative Humidity on Serum Prolactin and Growth Hormone in Heifers. Proc. Soc. Exp. Biol. Med. 151:623–626. doi:10.3181/00379727-151-39273.

Vallée, M., W. Mayo, F. Dellu, M. Le Moal, H. Simon, and S. Maccari. 1997. Prenatal stress induces high anxiety and postnatal handling induces low anxiety in adult offspring: correlation with stress-induced corticosterone secretion.. J. Neurosci. 17:2626–2636.

Verbeek, E., M. H. Oliver, J. R. Waas, L. M. Mcleay, D. Blache, and L. R. Matthews. 2012. Reduced cortisol and metabolic responses of thin ewes to an acute cold challenge in mid-pregnancy: Implications for animal physiology and welfare. PLoS One 7:e37315. doi:10.1371/journal.pone.0037315.

Viltart, O., and C. C. C. Vanbesien-Mailliot. 2007. Impact of prenatal stress on neuroendocrine programming. Sci. World J. 7:1493–1537. doi:10.1100/tsw.2007.204.

Vo, T., and D. B. Hardy. 2012. Molecular mechanisms underlying the fetal programming of adult disease. J. Cell Commun. Signal. 6:139–153. doi:10.1007/s12079-012-0165-3.

Weinstock, M. 2001. Alterations induced by gestational stress in brain morphology and behaviour of the offspring. Prog. Neurobiol. 65:427–451. doi:10.1016/S0301-0082(01)00018-1.

Wentzel, D., K. S. Viljoen, and L. J. J. Botha. 1979. Physiological and endocrinological reactions to cold stress in the Angora goat. Agroanimalia 11:19–22.

West, J. W. 2003. Effects of heat-stress on production in dairy cattle. J. Dairy Sci. 86:2131–2144. doi:10.3168/jds.S0022-0302(03)73803-X.

Wheelock, J. B., R. P. Rhoads, M. J. Vanbaale, S. R. Sanders, and L. H. Baumgard. 2010. Effects of Heat Stress on Energetic Metabolism in Lactating Holstein Cows. J. Dairy Sci. 93:644–55.

Wu, G., F. W. Bazer, J. M. Wallace, and T. E. Spencer. 2006. Intrauterine growth retardation: Implications for the animal sciences. J. Anim. Sci. 84:2316–2337. doi:10.2527/jas.2006-156.

Yadav, V. P., S. S. Dangi, V. S. Chouhan, M. Gupta, S. K. Dangi, G. Singh, V. P. Maurya, P. Kumar, and M. Sarkar. 2016. Expression analysis of NOS family and HSP genes during thermal stress in goat (*Capra hircus*). Int. J. Appl. Anim. Sci. 60:381–389. doi:10.1007/s00484-015-1035-5.

Yang, C.-H., H. Ko, L. C. Salazar, L. Llonch, X. Manteca, I. Camerlink, and P. Llonch. 2018. Pre-weaning environmental enrichment increases piglets' object play behaviour on a large scale commercial pig farm. Appl. Anim. Behav. Sci. 202:7–12. doi:10.1016/j.applanim.2018.02.004.

Yates, D. T., A. S. Green, and S. W. Limesand. 2011. Catecholamines mediate multiple fetal adaptations during placental insufficiency that contribute to intrauterine growth restriction : lessons from hyperthermic sheep. J. Pregnancy 2011:740408. doi:10.1155/2011/740408.

Yazdi, M. H., H. R. Mirzaei-Alamouti, H. Amanlou, E. Mahjoubi, A. Nabipour, N. Aghariazati, and L. H. Baumgard. 2016. Effects of heat stress on metabolism, digestibility, and rumen epithelial characteristics in growing Holstein calves. J. Anim. Sci. 94:78–89. doi:10.2527/jas2015-9364.

Young, B. A. 1981. Cold stress as it affects animal production. J. Anim. Sci. 52:154–163.

Young, B. A. 1983. Ruminant cold stress: effect on production. J. Anim. Sci. 57:1601–1607. doi:10.2527/jas1983.5761601x.

Zhu, M. J., M. Du, B. W. Hess, W. J. Means, P. W. Nathanielsz, and S. P. Ford. 2007. Maternal nutrient restriction upregulates growth signaling pathways in the cotyledonary artery of cow placentomes. Placenta 28:361–368. doi:10.1016/j.placenta.2006.04.005.