Universitat Autònoma de Barcelona

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DOCTORAL THESIS

Sandra González Luna

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Tesis presentada por **Sandra González Luna** y dirigida por el Dr. **Gerardo Caja López** y el Dr. **Ahmed A. K. Salama** del Departament de Ciencia Animal i dels Aliments de la Universitat Autònoma de Barcelona.

Bellaterra, 17 de junio de 2022

Dr. Gerardo Caja López

Dr. Ahmed A. K. Salama

A Dios por creer en mí cada día y darme la oportunidad de crecer en familia.

A mi madre Ana Luna, ejemplo fuerza y amor.

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SHORT BIOGRAPHY

Sandra González Luna was born in Mexico City (1986), she graduated in Veterinary Medicine and Animal Production at the National Autonomous University of Mexico (UNAM, 2011). Her interest in the field led her to further her knowledge and obtain a Master´s degree in Veterinary Medicine and Animal Production (UNAM, 2015) with honor. She was subsequently hired as a professor at the UNAM. In 2018, she obtained a scholarship from CONACYT, Mexico to pursue her PhD studies at the Autonomous University of Barcelona (UAB). During this period, she developed research skills related to statistical analyses, research methodology and writing research. She attended national and international meetings and earned awards for the best presentation on sheep research in the XX International Congress of SEOC, and the best poster in the Seminar for PhD students of animal Production and Food Science Programs at the UAB.

SCIENTIFIC DISSEMINATION DURING PhD

International Conference symposia

S. Serhan, **S. González-Luna**, B. Chaalia, X. Such, A. A. K. Salama, and G. Caja. 2020. Metabolic and productive characteristics of sensitive and heat-tolerant phenotypes of Murciano-Granadina dairy goats. American Dairy Science Association (ADSA), Virtual Annual Meeting. J. Dairy Sci. 103:44.

B. Chaalia, S. Serhan, **S. González-Luna**, X. Such, A. A. K. Salama, and G. Caja. 2020. Metabolic and productive characteristics of sensitive and heat-tolerant phenotypes in dairy sheep. American Dairy Science Association (ADSA), Virtual Annual Meeting. J. Dairy Sci. 103:23.

A. Castro-Costa, J. Oliver**, S. González-Luna**, B. Chaalia, S. Serhan, A.A.K. Salama, C. Ferrer and G. Caja. 2020. Rumen temperature of lactating dairy ewes under different ambient

conditions by bolus sensors. Page 199 in Proc. 71st Annual Virtual Meeting of European Federation of Animal Science (EAAP). Book of abstracts No. 26.

Chaalia, S. Serhan, **S. Gonzalez-Luna**, X. Such, A.A.K. Salama and G. Caja. 2020. Responses of dairy ewes to heat stress according to their phenotypical heat tolerance. Page 424 in Proc. 71st Annual Virtual Meeting of the European Federation of Animal Science (EAAP). Book of abstracts No. 26.

A.A.K. Salama, **S. González-Luna**, M. Ramon, S. Serhan, X. Such, C. Díaz, M. J. Carabaño and G. Caja. 2021. Liver trancriptomics of sensitive and heat- tolerant dairy goat phenotypes. Page 223 in Proc. 72nd Annual Meeting of European Federation of Animal Science (EAAP). Book of abstracts No. 27. Davos, CH.

M. Ramon, **S. González-Luna**, S. Serhan, A.A.K. Salama, A. Molina, M. Sánchez, C. Díaz, X. Such, G. Caja and M. J. Carabaño. 2021. Individual differences in the physiological response to heat stress of dairy goats. Page 134 in Proc. 72 nd Annual Meeting of European Federation of Animal Science (EAAP). Book of abstracts No. 27. Davos, CH.

National Conference symposia

Caja, G., Elhadi, A., **González-Luna, S.**, Cedeño, M., Chaalia, B., Serhan, S., Salama, A.A.K. y Such, X. 2019. Características termofisiológicas y variación de la respuesta a un reto de estrés por calor en un rebaño de ovejas manchegas. Pages 33−35 in Proc. XVIII Jornadas sobre Producción Animal, Asociación Interprofesional para el Desarrollo Agrario (AIDA), Zaragoza, ES.

González-Luna, S., Cordón, L., Salama, A.A.K., Such, X., Albanell, E., Contreras-Jodar, A., De Lucas, J. y Caja, G. 2019. Comparación de estrategias de esquileo en ovejas lecheras. Pages 565−571 in Proc. XX Congreso Internacional de la Sociedad Española de Ovinotecnia y Caprinotecnia (SEOC), Córdoba, ES.

González-Luna, S., Serhan, S., Chaalia, B., Such, X., Salama, A.A.K. y Caja, G. 2021. Respuestas metabólicas y productivas de fenotipos sensibles y tolerantes al calor de cabras

lecheras de raza Murciano-Granadina. Page 263 in Proc. XIX Jornadas sobre Producción Animal, Asociación Interprofesional para el Desarrollo Agrario (AIDA). Zaragoza, ES.

González-Luna, S., Chaalia, B., Serhan, S., Such, X., Caja, G. y Salama, A.A.K. 2021. Respuestas metabólicas y productivas de fenotipos sensibles y tolerantes al calor de ovejas lecheras de raza Manchega. Page 264 in Proc. XIX Jornadas sobre Producción Animal, Asociación Interprofesional para el Desarrollo Agrario (AIDA). Zaragoza, ES.

LIST OF ABBREVIATIONS

ATP	Adenosine triphosphate
ALT	Alanine amino transferase
ALP	Alkaline phosphatase
BHB	β-hydroxybutyrate
BCS	Body condition score
BW	Body weight
CO	Control
F45	Curd firmness at 45 min
DM	Dry matter
ER	Endoplasmic reticulum
ECM	Energy corrected milk
GO	Gene ontology
GH	Growth hormone
HSP	Heat shock protein
HS	Heat stress
IPCC	Intergovernmental Panel on Climate Change
LC	Lacaune breed
LPS	Lipopolysaccharide
LFC	Log_2fold change
MN	Manchega breed
NRC	National Research Council
NEFA	Non-esterified fatty acids

PRL Prolactin

ROS Reactive oxygen species

RT Rectal temperature

RH Relative humidity

RCT Rennet coagulation time

RR Respiratory rate

S Sensitive

SBB Shorn at d 100 of pregnancy

S100 Shorn before breeding

SCC Somatic cell count

THI Temperature Humidity Index

TN Thermoneutral

T4 Thyroxine

T Tolerant

T3 Triiodothyronine

UPR Unfolded protein response

ABSTRACT

Shearing effects during pregnancy and productive and transcriptomic responses to heat stress according to phenotype (sensitive vs. tolerant) in dairy sheep

Two experiments were carried out to assess the effect of shearing strategies in dairy ewes under hot season and to evaluate the phenotypic and cellular responses to heat stress (HS) in tolerant and heat-sensitive dairy ewes. In **Exp. 1**, 73 pregnant ewes (MN, Manchega, n = 43; LC, Lacaune, n = 30) were divided into 3 balanced groups and randomly assigned to the experimental treatments (unshorn, CO; shorn before breeding, SBB; and shorn at d 100 of pregnancy, S100) in summer season. The S100 reduced the respiratory frequency (−37%) of both breeds on average. Responses to shearing varied according to breed; MN ewes (SBB and S100) had greater glycemia at lambing (86%) than CO, but did not vary in LC ewes, and no effects were detected on plasmatic insulin, BHB, and NEFA values in either breed. No effects were detected on lamb birth weight and growth, colostrum, or milk yield in either breed. During suckling, S100 MN ewes had more milk protein (6%) and casein (6%) compared with CO ewes, whereas S100 LC had more milk solids (8%) and fat (8%) than SBB ewes. During late pregnancy, the S100 ewes had greater BCS than CO and SBB in both breeds. Milk of S100 MN ewes had longer coagulation time (9%) than CO, but it was shorter (−8%) in LC ewes. In **Exp. 2.1**, 24 ewes in late lactation were submitted to a short heat tolerance test (90 min at 35.6ºC and 43% humidity; THI = 85). According to change ratio (CR = after/before test) of rectal temperature (RT) and respiratory rate (RR), a subset of 10 ewes differing in CR phenotype (sensitive; S, n = 5, tolerant; T, n = 5) were identified. They were enrolled in a crossover design of 2 periods (3 wk each) and 2 climatic conditions: 1) thermo-neutral (TN; 15 to 20°C, THI = 65-63 day-night), and 2) HS (day, 37°C, THI = 87; night, 30°C, THI = 79). The HS ewes increased RT (0.54ºC a.m. and p.m.), RR (126 and 227% at a.m. and p.m., respectively), water intake (35%), and decreased feed intake (−20%) compared to TN ewes. Milk yield did not vary, but milk fat (−14%) and protein (−17%) were reduced in HS ewes. Ewes rose plasma NEFA (74%), prolactin (415%), and creatinine (10%) without changes in glucose and insulin values under HS. Comparing T and S phenotypes, no changes were detected in feed and water intakes, milk yield and composition, or blood indicators. However, T vs. S phenotypes had different magnitude of increase in RT (0.47 vs. 0.61ºC at p.m.) with lower increment in water consumption (24 vs. 45%) in response to HS. In **Exp 2.2**, the T and S ewes underwent liver biopsy at the end of each experimental period (Exp. 2.1) for RNA-seq analysis. Comparing HS vs. TN, ewes downregulated 39 genes associated with muscle contraction, transition between fast and slow fiber, and sarcomere organization. Under HS, T vs. S ewes downregulated 893 genes (e.g., SPP1, LEPR) related to endocytosis, N-glycan biosynthesis, and complement and coagulation cascades pathways. They also upregulated 425 genes related to fatty catabolism (UCP3) and insulin signaling resistance (IP6K3). In conclusion, shearing ewes at late pregnancy is a recommended practice to alleviate HS impact without negative effects on lactational performances. Additionally, HS causes metabolic adjustments in dairy ewes accompanied by different cellular responses between the tolerant and sensitive phenotypes.

RESUM

Efectes de l'esquilada durant la gestació i respostes productives i transcriptòmiques a l'estrès per calor segons el fenotip (sensible vs. tolerant) en ovelles lleteres

L'objectiu va ser avaluar l'efecte de les estratègies d'esquilament en ovelles lleteres durant l'època de calor, i les respostes fenotípiques i cel·lulars a l'estrès per calor (HS) en ovelles lleteres tolerants i sensibles a la calor. A l'**Exp. 1**, 73 ovelles gestants (MN, Manxega, n = 43; LC, Lacaune, n = 30) repartides en 3 grups balancejats es van assignar aleatòriament als tractaments experimentals (sense esquilar, CO; esquilat abans de la munta, SBB, i esquilat als 100 d de gestació, S100) durant l'estiu. El grup S100 va reduir (−37%) la freqüència respiratòria a les dues races. La resposta a l'esquilat va variar segons la raça: en MN, els grups SBB i S100 van tenir una glucèmia més alta al part (86%) que el grup CO; però no es van observar diferències entre grups a les LC. Tampoc es van detectar efectes sobre els valors d'insulina plasmàtica, BHB i NEFA, el pes al naixement i el creixement dels xais, en la composició del calostre ni en la producció i composició de la llet en cap de les dues races. Durant la lactància dels xais, la llet de les ovelles S100 MN va presentar un major contingut de proteïna (6%) i caseïna (6%) que a les ovelles CO, mentre que la llet de les S100 LC contenia més sòlids totals (8%) i greix (8%) que les ovelles SBB. Al final de la gestació, en les dues races les S100 van tenir un major BCS que les CO i SBB. En llet de les ovelles MN, les S100 van tenir un temps de coagulació més llarg (9%) que el CO, però en el cas de les LC aquest va ser més curt (−8%). A l'**Exp. 2.1**, 24 ovelles al final de la lactació van ser sotmeses a una prova curta de tolerància a la calor (90 min a 35,6ºC i 43% d'humitat; THI = 85). En base a la ràtio de canvi (CR = després/abans de la prova) de la temperatura rectal (RT) i la freqüència respiratòria (RR), es van seleccionar 10 ovelles que diferien al fenotip CR (sensible; S, n = 5, tolerant, T, n = 5). La prova va seguir un disseny creuat de 2 períodes (3 setmanes cadascun) i 2 condicions climàtiques: 1) termoneutre (TN; 15 a 20°C, THI = 65-63 dia-nit), i 2) HS (dia, 37°C, THI = 87; nit, 30°C, THI = 79). Les ovelles HS van augmentar la TR (0.54ºC a.m. i p.m.), la RR (126 i 227% a.m. i p.m., respectivament) i el consum d'aigua (35%), i van disminuir el consum d'aliment (−20%) respecte les TN. La producció de llet no va variar, però es va reduir el greix de la llet (−14%) i la proteïna (−17%) en les HS. Les HS van incrementar els NEFA plasmàtics (74%), la prolactina (415%) i la creatinina (10%) sense canvis en els valors de glucosa i insulina. Comparant els fenotips T i S, no es van detectar canvis en el consum d'aliment i aigua, la producció i composició de la llet o als indicadors sanguinis. Tot i això, els dos fenotips van tenir diferent magnitud d'increment en resposta a HS a la RT (T, 0.47 vs. S, 0.61ºC a la tarda) amb un menor increment en el consum d'aigua (T, 24 vs. S, 45%). A l'**Exp 2.2**, al final de cada període experimental (Exp. 2.1) es van biosiar el fetge les ovelles T i S per a l'anàlisi de l'RNA-seq. Les HS vs. TN van regular a la baixa 39 gens associats amb la contracció muscular, la transició entre fibra ràpida i lenta i l'organització del sarcòmer. Sota HS, les T vs. S van regular a la baixa 893 gens (p. ex., SPP1, LEPR) relacionats amb l'endocitosi, la biosíntesi de N-glicans i les vies de les cascades del complement i la coagulació; i a l'alça 425 gens relacionats amb el catabolisme gras (UCP3) i la resistència a la senyalització d'insulina (IP6K3). En conclusió, l'esquilada de les ovelles al final de la gestació és una pràctica recomanada per mitigar l'impacte de l'HS sense efectes negatius en el rendiment de la lactanció. A més, l'HS provoca ajustaments metabòlics en ovelles lleteres acompanyats de diferents respostes cel·lulars entre els fenotips tolerants i sensibles.

RESUMEN

Efecto del esquileo durante la gestación y respuestas productivas y transcriptómicas al estrés por calor según el fenotipo (sensible vs. tolerante) en ovejas lecheras

Se realizaron dos experimentos para evaluar el efecto de las estrategias de esquileo en ovejas lecheras durante la época de calor y para evaluar las respuestas fenotípicas y celulares al estrés por calor (HS) en ovejas lecheras tolerantes y sensibles al calor. En el **Exp. 1**, 73 ovejas gestantes (MN, Manchega, n = 43; LC, Lacaune, n = 30) se dividieron en 3 grupos balanceados y se asignaron aleatoriamente a los tratamientos experimentales (sin esquilar, CO; esquilado antes de la monta, SBB; y esquilado a los 100 d de gestación, S100) durante la época de verano. El grupo S100 redujo de media un 37% la frecuencia respiratoria en ambas razas. En ovejas MN, los grupos SBB y S100 tuvieron mayor glucemia al parto (86%) que el grupo CO, sin diferencias en las ovejas LC. No se detectaron efectos en insulina, BHB y NEFA, peso al nacimiento de los corderos, y producción de leche en ninguna de las dos razas. Durante la lactancia de los corderos, la leche de las ovejas S100 MN tuvo mayor proteína (6%) y caseína (6%) que las CO, mientras que la leche de las S100 LC tuvo más sólidos totales (8%) y grasa (8%) que las SBB. Al final de la gestación, las ovejas S100 tuvieron mayor BCS que las CO y SBB en ambas razas. La leche de las ovejas S100 MN tuvo un tiempo de coagulación más largo (9 %) que el CO, pero en el caso de las LC, éste fue más corto (−8 %). En el **Exp. 2.1**, 24 ovejas al final de la lactación fueron sometidas a una prueba corta de tolerancia al calor (90 min a 35.6ºC y 43% de humedad; THI = 85). En base a la ratio de cambio (CR = después/antes de la prueba) de la temperatura rectal (RT) y la frecuencia respiratoria (RR), un subgrupo de 10 ovejas que diferían en el fenotipo CR (sensible; S, n = 5, tolerante; T, n = 5) fueron identificados. Las ovejas fueron sometidas a un experimento con diseño cruzado de 2 períodos (3 semanas) y 2 condiciones climáticas: 1) termo-neutro (TN; 15 a 20°C, THI = 65-63 día-noche), y 2) estrés por calor (HS; día, 37°C, THI = 87; noche, 30°C, THI = 79). Las ovejas HS aumentaron la TR (0.54ºC a.m. y p.m.), RR (126 y 227% a.m. y p.m., respectivamente), el consumo de agua (35%) y disminuyeron el consumo de alimento (−20%) en comparación con las ovejas TN. La producción de leche no varió, pero la grasa de la leche (−14%) y la proteína (−17%) se redujo en las ovejas HS. Las ovejas en HS incrementaron los NEFA (74%), la prolactina (415%) y la creatinina (10%) sin cambios en glucosa e insulina. Comparando los fenotipos T y S, no se detectaron cambios en el consumo de alimento y agua, la producción y composición de la leche o en los indicadores sanguíneos. Sin embargo, los fenotipos T vs. S tuvieron diferente magnitud de incremento en respuesta a HS en la RT (0.47 vs. 0.61ºC p.m.) y consumo de agua (24 vs. 45%). En el **Exp 2.2**, las ovejas T y S se sometieron a una biopsia de hígado al final de cada período experimental (Exp. 2.1) para el análisis del RNA-seq. Al comparar HS con TN, las ovejas desactivaron 39 genes asociados con la contracción muscular, la transición entre fibra rápida y lenta. Bajo HS, las ovejas T vs. S desactivaron 893 genes (p. ej., SPP1, LEPR) relacionados con la endocitosis, la biosíntesis de N-glicanos y las vías de las cascadas del complemento. También activaron 425 genes relacionados con el catabolismo graso (UCP3) y la resistencia a la señalización de insulina (IP6K3). En conclusión, el esquileo de las ovejas al final de la gestación es una práctica recomendada para mitigar el impacto del HS sin efectos negativos en el rendimiento de la lactación. Además, el HS provoca ajustes metabólicos en ovejas lecheras acompañados de diferentes respuestas celulares entre los fenotipos tolerantes y sensibles.

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Literature review

1.1 Introduction

In general terms and according to FAOSTAT (2020), dairy sheep accounts for 20.3% of total sheep population, and are mainly located in Asia (51.3%), followed by Africa, Europe, and America (Table 1.1). In the same year, the total sheep milk production reached 10.6 Mt, of which 46.3% were produced in Asia, 29.3% in Europe, 23.5% in Africa, and less than 1% in America. However, the calculated individual milk yield was more than 2-fold greater in Europe (99.3 kg/head; Table 1.1) than world average milk yield, showing the growth potential of the world dairy sheep sector, as previously described by Pulina et al. (2018). This milk yield performance per ewe in Europe is reflected in its cheese production, which accounted for around 57% of total world cheese production (Figure 1.1).

More specifically, it should be stressed that countries bordering the Mediterranean basin and Black Sea, produce half (50.5%; 5.4 thousands of tons) of total sheep milk (FAOSTAT, 2020), where consuming dairy sheep products is a long tradition (Milan et al., 2014) and dairy sheep farms are more concentrated. In this region the three main producers are Turkey (1207 Mt, 59 kg/head), Greece (945 Mt, 151 kg/head) and Syria (706 Mt, 67 kg/head). Nevertheless, when comparing by milk yield (kg/head), Spain (556 Mt, 236 kg/head), France (326 Mt, 203 kg/head) and Greece are the top yielders (FAOSTAT, 2020).

Table 1.1. Overview of dairy sheep statistics

	Total sheep		Dairy sheep		Milk		Yield
Continent	Million head	%	Million head	%	Mt	%	kg/head
Asia	547	43.3	132	51.3	4.9	46.3	37.3
Africa	418	33.1	91	35.5	2.5	23.5	27.4
Europe	125	9.9	31	12.2	3.1	29.3	99.3
America	83	6.6	3	1.1	0.1	0.9	32.6
Oceania	90	7.1	-	-	-	-	-
Total	1263	100	257	100	10.6	100	41.3[1]

[1]Mean value rather than the total. Source: FAOSTAT (2020).

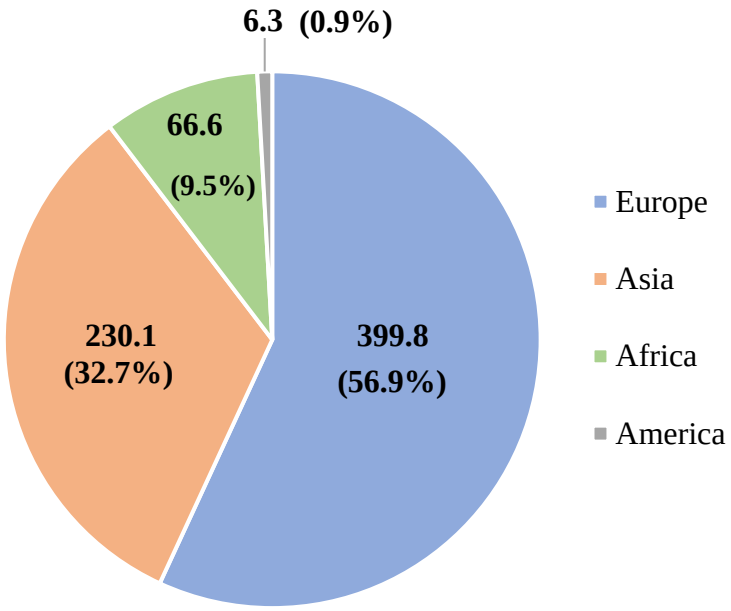


Figure 1.1 Sheep cheese production by continent [thousand tons and (%)]. Source: FAOSTAT, 2019.

The aforementioned data clearly show the importance of dairy sheep production in the Mediterranean basin, a region that is characterized by warm to hot, dry summers, and mild to cool, wet winters, with specific regional characteristics (Ramón et al., 2016). In the context of climate change, predictions indicate that Mediterranean basin is one of the regions where higher temperature increase, coupled with reduced precipitations, are expected during hot seasons, (Segnalini et al., 2013. In this scenario livestock will be exposed to heat stress (HS) and more vulnerable to its negative effects depending on species, breed, physiological state, production level, management and production systems, level of insulation (e.g., wool cover) and nutritional status (Silanikove 2000; Marai et al., 2007; Sejian et al., 2018), among other factors. For example, goats, followed by sheep are considered less sensitive to HS than cattle (Lu 1989; Silanikove, 2000). In addition, dairy cattle are especially more susceptible to HS than beef cattle due to the overall increase in endogenous heat production (Bernabucci et al., 2010).

Heat stress is a major concern in livestock because it jeopardizes animal welfare and compromises several productive variables, including milk yield and composition, growth,

reproduction, and carcass features (Baumgard and Rhoads, 2013). Hence, HS is being a threat for livestock-based food security in many parts of the world (Sejian et al., 2018).

Some strategies to ameliorate the impact of HS on livestock include: 1) physical modification of the environment, 2) genetic development of heat-tolerant breeds, and 3) improved nutritional management practices (Beed and Collier, 1986; Salama et al., 2014, 2020).

According to Renaudeau et al. (2012), modifying the environment should be oriented to prevent or limit the degree of HS (e.g., shade provision, cooling systems) or enhance animal heat losses (e.g., ventilation, sprinkles). Regarding the latter, in the case of woolled sheep breeds (all dairy sheep have this feature), shearing is a management practice that could contribute to improve heat-loss capacities during hot seasons given that wool cover causes less effectiveness of sweating in sheep (Marai et al., 2007). Evidence of the improved heat dissipation mechanisms in ewes because of shearing, in terms of lower rectal temperature and respiratory rates has been reported in dairy ewes shorn at late pregnancy during summer season (Leibovich et al., 2011) and lactating during winter (Elhadi et al., 2019). However, shearing during hot conditions in pregnant ewes and its effects on performance during the subsequent lactation and on the offspring have been less studied in dairy sheep.

The breeding of thermo-tolerant animals as another mitigation strategy to cope with HS, requires the identification of thermotolerant animals by assessing their response to HS (e.g., thermo-physiological traits, performance, metabolic indicators). High genetic variability between and within breeds, and even between individuals within species or breed, suggest that it is feasible to select for tolerance to HS (Renaudeau et al., 2012). However, there is evidence of antagonism between milk yield and heat tolerance in dairy animals (West, 2003; Finocchiaro et al., 2005), which makes this topic more complex.

In this sense, transcriptomics has become a powerful tool for analyzing the relationship between genotype and phenotype (Lu et al., 2019), which can provide deeper insight of HS impact. The identification of differentially expressed genes and potential mechanisms involved in the metabolic regulation of heat-tolerant dairy ewes through transcriptomic profiling of liver tissue, would enhance the understanding of HS response and allow improvements of heat tolerance in dairy ewes through genetic selection.

1.2 Climate change

Climate change is one of the main challenges in the current century and is defined as the long-term alterations in weather conditions (temperature, wind, and rainfall characteristics) of a specific region (Bernabucci et al., 2010). Global food security, which is based on agriculture production and livestock, will be more affected by climate change, especially by global warming (Silanikove and Koluman 2015; Bernabucci, 2019). Unfortunately, the predictions indicate that by the end of the 21st century (2081-2100), the global mean surface temperature will increase 0.3 to 4.8 °C relative to 1986-2005 period as shown in Figure 1.2 (IPCC, 2014). If this warmer scenario continues as predicted, more strategies addressed to alleviate the impact of HS on livestock are necessary, which undoubtedly requires understanding of phenotypic responses of animals (e.g., physiology, behavior, productive performance, metabolism, etc.), coupled with the underlying genetic mechanisms (e.g., gene expression patterns and networks).

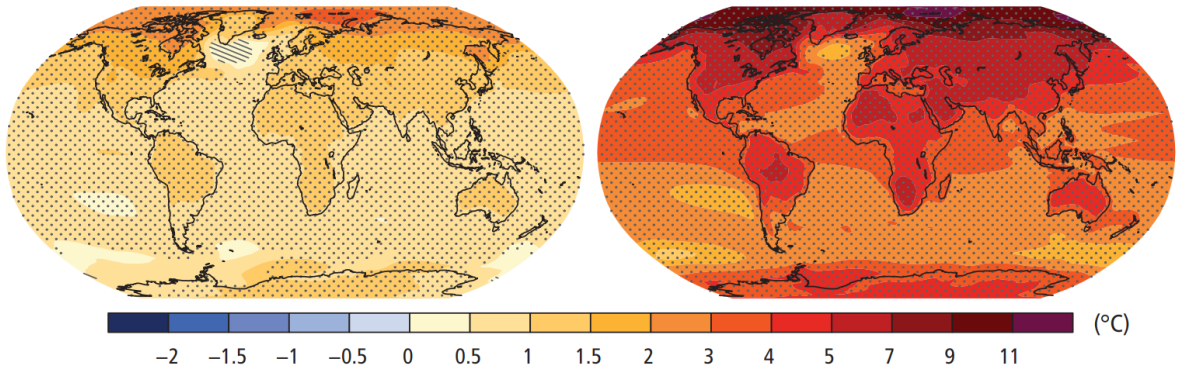


Figure 1.2. Change in average surface temperature (1986-2005 to 2081-2100). IPCC (2014).

1.3 Thermoregulation in sheep and heat stress

Sheep as most mammals, are homeotherms that maintain core body temperature within a narrow range (Joy et al., 2020) by activating thermoregulatory mechanisms to balance heat production and heat losses (Renaudeau et al., 2012. The rectal temperature in sheep ranges between 38.3-39.9 °C, under thermoneutral conditions (Marai et al., 2007).

Thermoregulation is a neural process that connects information from thermal environment (external and internal) with autonomic efferent responses that in turn control cellular metabolism and endocrine system (Collier and Gebremedhin, 2015), as shown in Figure 1.3.

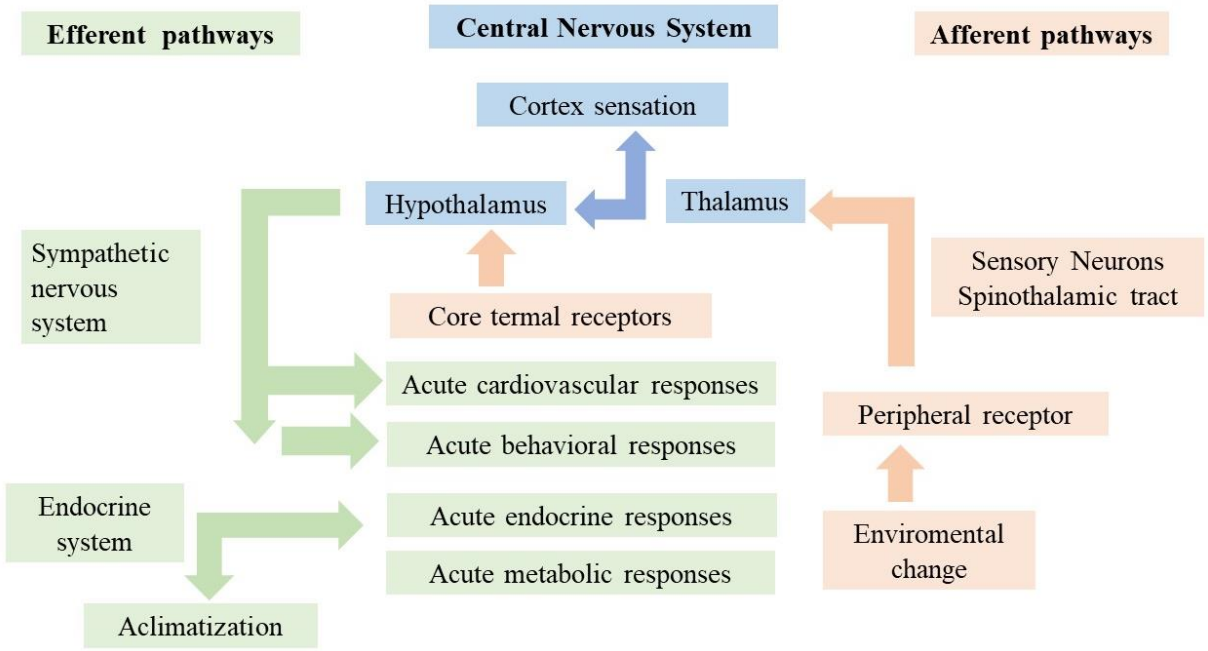


Figure 1.3. Neural integration scheme of environmental stress with animal responses (Collier et al., 2018).

The dynamic process of thermal balance requires heat exchange to maintain animals in their thermoneutral zone, in which animals spend the minimum energy in the thermoregulatory process, and thus they devote most of it for growth and production purposes (Spiers 2012; Collier et al., 2018). The complex balance of thermogenesis and thermolysis in determining core body temperature and thermoneutral zone is shown in Figure 1.4.

In high ambient temperatures, heat dissipation occurs by sensible pathways (conduction, convection, and radiation), but if thermal environment exceeds the animal's body temperature additional energy is required, sensible routes decrease, and evaporative avenues for heat losses (sweating and panting) are activated (Lu, 1989; Collier et al., 2018).

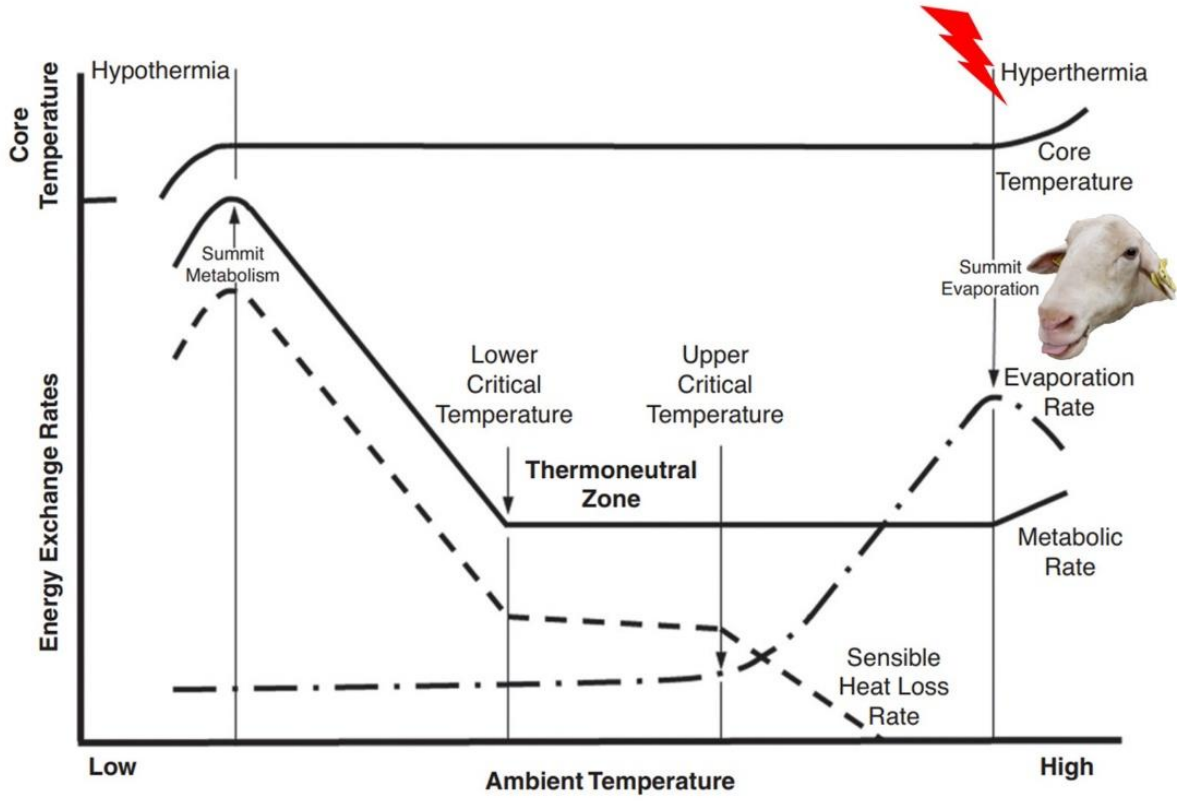


Figure 1.4. Limits of homeothermy are identified with summit metabolic and evaporation rates. The thermoneutral zone is displayed by lower and upper critical temperatures.(Adapted from Spiers, 2012).

In general, the boundaries of thermoneutral zone are affected by many factors including animal species, breed, age, production level, body weight, thermal insulation, nutrition, humidity, air movement, and housing conditions among others (Yousef, 1985).

In the case of most breeds of sheep, fleece (e.g., thickness and extension), confers more insulation capacity, which greatly influence the limits of their thermoneutral zone (NRC, 1981; Lu, 1989). Consequently, panting in woolled sheep becomes the most important way for heat dissipation, and sweating is much less effective (Marai et al., 2007).

1.4 Measuring heat stress level

Animals undergo HS when heat load (internal and from the environment) surpasses their dissipation capacity, leading to core body temperature increase above the range specified for normal activity (Bernabucci et al., 2010). Heat stress impact on animals could be measured by assessing their phenotypic responses, such as rectal temperature, respiratory rate, metabolic

indicators, and productive performance (Sejian et al., 2019). Such phenotypic responses are determined by gene expression and gene networks, both coordinated in time and space (Gracey, 2007). In this framework, body temperature and respiratory rate are considered excellent indicators of HS, however, recording these individual data is not feasible on a large scale in farm conditions (Finocchiaro et al., 2005; Salama et al., 2016). Heart rate is an additional good indicator, which typically increases in heat exposed animals and results in a blood flow deviation from internal body towards the subcutaneous layer (Wojtas et al., 2014).

As an alternative to animal indicators, a variety of environmental indices could be used for HS monitoring, including the most widely used temperature-humidity index (THI) (Dikmen and Hansen, 2009). The THI represents the merged effects of temperature and moisture of the air (Bohmanova et al., 2007) and data available from meteorological stations confer a practical usage of THI. However, THI as environmental indicator, is less accurate monitoring HS than animal-based indicators (Galán et al., 2018) because it does not consider other factors such as wind speed, solar radiation, age, breed or productive level of animals (Hammami et al., 2013; Serradilla et al., 2017).

Several THI equations, differing in dry bulb temperature and air moisture weightings, have been proposed due to the differences in sensitivity to ambient temperature and humidity among species (Bohmanova et al., 2007). Initially, THI was developed by Thom (1959) as a human HS index but has been extensively used for monitoring HS in different animal species, especially cattle (Bianca 1962; NRC, 1971; Yousef et al., 1985 and Mader et al., 2006).

The differences in detecting HS according to THI equation, and specific climatic factors of each region are evidenced in several studies, which further complicate scientific literature comparisons, as pointed out by Ramon et al. (2016) who highlighted the need of developing a THI adapted to specific climatic regions for sheep. Thus, Marai et al. (2007) proposed THI thresholds in sheep depending on whether ambient temperature is measured in °F or °C (Table 1.2).

Table. 1.2. The temperature-humidity index (THI) thresholds proposed for sheep (Marai et al., 2007).

Heat stress class	Equation using °F[1]	Equation using °C[2]
Absence	THI <82	THI < 22.2
Moderate	82 ≤ THI < 84	22.2 ≤ THI < 23.3
Severe	84 ≤ THI < 86	23.3 ≤ THI < 25.6
Extreme	THI ≥ 86	THI ≥ 25.6

[1]THI = db °F − {(0.55 − 0.55 RH)·(db °F − 58)}, where db °F is the dry bulb temperature in °F and RH is the relative humidity (RH%)/100.
[2]THI = db °C − {(0.31 − 0.31 RH)·(db °C − 14.4)}, where db °C is the dry bulb temperature in °C and RH is the relative humidity (RH%)/100.

Finocchiaro et al. (2005) reported that daily milk and fat-protein yield started to decline above a THI of 23 in Valle del Belice ewes, losing 62.8 g and 8.9 g per ewe, respectively for each unit increment of THI. The equation used by Finocchiaro et al. (2005) was: THI = {T – [0.55 × (1 – RH)] × (T – 14.4)}, where T is the maximum temperature in °C and RH is relative humidity %. Using the same equation, Ramón et al. (2016) reported production losses that ranged between 1 to 5 g/d (milk yield) and 0.1 to 0.3 g/d (fat and protein) per °C (or THI unit) above THI of 18 in Manchega ewes.

Regarding dairy cows and goats, Table 1.3 shows the THI thresholds for milk yield decline.

Table 1.3. Heat stress classes according to temperature-humidity index (THI) for dairy goats and cows (from Silanikove and Koluman 2015).

THI category[1]	Heat stress class	Dairy cows	Dairy goats
Normal	No effect on milk yield	THI < 74	THI < 80
Alert	Modest effect on milk yield	74 ≤ THI < 79	80 ≤ THI < 85
Danger	Severe effect on milk yield	79 ≤ THI < 84	85 ≤ THI < 90
Emergency	Death risk	THI ≥ 84	THI ≥ 90

[1]Based on Livestock Weather Safety Index using THI = 0.8tdb + RH (tdb – 14.4) + 46.4, where tdb is dry bulb temperature in °C and RH is relative humidity in decimal form (Thom, 1959).

1.5 Shearing as a management practice that modify thermoneutral zone boundaries

The wool cover provides thermal insulation and reduces heat loss since it hinders water evaporation from the body or sweating rate (Wojtas et al., 2014; McMagnus et al., 2020), when the animal is exposed to hot conditions. Therefore, shearing shifts the thermoneutral zone boundaries by expanding the lower critical temperature which in turn induces adaptive responses to maintain homeostasis (Aleksiev, 2008).

According to production system and climatic conditions, the management practice of shearing is implemented once a year, generally at the beginning of summer in many Mediterranean countries (Dyrmundsson, 1991). In Spain, shearing is typically performed on mid-May, prior to mating and starting traditional grazing on cereal stubbles or transhumance (Elhadi et al., 2019). This is done before summer arrival to improve heat dissipation capacity during hot season given that pregnant and lactating ruminants are considered more sensitive to heat stress effects (Silanikove, 1992). In dairy ewes, shearing is also done during pregnancy to enhance ewe's fitness before lambing in winter and, as reported by Elhadi et al. (2019), at mid-lactation for milk hygiene and milking easiness, without negative effects on milk performance in terms of yield and composition.

Pregnancy shearing has received special interest since Rutter et al. (1971, 1972) reported increased birthweight and survival of lambs born from shorn pregnant ewes in winter season. Table 1.4 summarizes the main effects of pregnancy shearing in different breeds and seasons. Lamb birth weight increases (9 to 32%), but some studies indicate no effect. This variation could be caused by the litter size, the influence of ewe potential (e.g., breed giving lambs of low birthweight) and resources availability (e.g., adequate body reserves or nutrition), as reviewed by Kenyon et al. (2003). With regard to the lactational performances, the results indicate no effects or alternatively, higher milk yield (7 to 41%), milk fat (8 to 11%), milk protein (3 to 11%) and colostrum lactose (27%) contents, associated or not with a improved feed intake. Additionally, the shearing effects on curdling properties of milk which constitutes a key topic when it comes to cheese making, have not been reported in pregnant ewes so far.

As stated above, the results of pregnancy shearing vary according to timing (e.g., days to lambing), purpose (e.g., meat, wool, or dairy), litter size (e.g., single, or twin-bearing ewes), season (e.g., summer or winter) and farming conditions (e.g., housed or grazing). Albeit the effects of pregnancy shearing have been widely studied, which is clear is the scarce research available regarding the lactational and offspring performances of dairy ewes, because of improved heat dissipation mechanisms to cope with heat load.

Table 1.4. Summary of main effects of pregnancy shearing on ewe performances and offspring (SH = shorn, CO = control).

Reference	Breed (ewes)	Season	Ewe state	Treatments	Main effects (shearing vs. control)			
					Body reserves	DMI	Milk	Lambs
Symonds et al. (1986)	Leicester × SWE (n = 18)	Winter	Late pregnancy	SH vs. CO	No effect	No effect	n/d	BBW (+16%)
.Knight et al. (1993)	Dorset (n = 60)	Winter	Late pregnancy	SH vs. CO	No effect	+46%	No effect on yield. Fat (+8%), protein (+11%)	n/d
Dabiri et al. (1996)	BL × Romney (n = 60)	Autumn spring	Late pregnancy	SH vs. CO	No effect	+14%	No effect	No effect
Morris and McCutcheon (1997)	BL × Romney (n = 214)	Winter	Mid-late pregnancy	SH_{70}, SH_{100}, SH_{130} vs. CO	No effect on BW, BCS (n/d)	n/d	n/d	BBW twins: SH_{70} (+14%), SH_{100} (+9%), SH_{130} (+7%). No effect on singletons
Avondo et al. (2000)	Comisana (n = 28)	Summer	Mid pregnancy	SH vs. CO	No effect	+20%	No effect	No effect
Revell et al. (2000)	Coopworth cross (n= 30)	Winter	Mid pregnancy	SH vs. CO	n/d	T (+44%) S, no effect	n/d	BBW twins (+26%). No effect on singletons
Cam and Kuran (2004)	Karayaka (n= 46)	Winter	Late pregnancy	SH vs. CO	No effect	n/d	Yield at 75 d of suckling (+41%)	BBW singletons (+19%) No effect on twins
Banchero et al. (2010)	Corriedale (n = 57)	Winter	Mid-late pregnancy (S)	SH_{70}, SH_{120} vs. CO	n/d	n/d	Colostrum. No effect on yield and composition	No effect on BBW. Sucking[1]: SH_{70} (78%), SH_{120} (61%) vs. CO (21%)
	Corriedale (n = 57)	Winter	Mid-late pregnancy (T)	SH_{70}, SH_{120} vs. CO	n/d	n/d	Colostrum. No effect on yield, lactose SH_{120} vs. CO (+27%)	BBW: SH_{70} (+26%), SH_{120} (+10%). Suckling[1]: SH_{70} (67%), SH_{120} (63%) vs. CO (22%)
Sphor et al. (2011)	Polwarth (n = 10)	Winter	Early pregnancy	SH vs. CO	BWL (+10%), No effect on BCS	n/d	Yield in suckling (+22%). No effect on composition	BBW (+32%). Weaning BW (+19%)
Leibovich et al. (2011)	Assaf (n = 150)	Summer	Late pregnancy	SHC[2] vs. CO	n/d.	+8%	+7%. ECM (+10%), fat (+11%), protein (+3%)	BBW (+9%)
García-Rodríguez et al. (2012)	Latxa (n = 50)	Winter	Late pregnancy	SH vs. CO	No effect on BW	+15%	No effect	No effect

BBW = birth body weight of lambs; BCS = body condition score; BL = Border Leicester; BW = body weight; BWL = body weight of the ewes at lambing; DMI = dry matter intake; S = single-bearing ewes; SH_{days} = time of pregnancy at shearing; SWE = Swaledale; T = twin-bearing ewes; [1]Sucked lambs on 1st hour of birth; [2]Shearing plus cooling; n/d = not determined.

1.6 Acclimatization

The acclimatization process to HS includes two consecutive phases: 1) acute or short term, and 2) chronic or long term (Johnson and Vanjonack, 1976; Horowitz, 2002; Garrett et al., 2009). The acute phase includes not only a shock response at the cellular level (Carper et al., 1987; Sonna et al., 2002), but also endocrine, physiological, and metabolic responses at the systemic level. In contrast, the chronic phase results in acclimation to the stressor and involves the reprogramming of gene expression and metabolism (Horowitz, 2002; Collier et al., 2006). The time required to complete both phases is weeks rather than days (Collier et al., 2006).

1.7. Responses of ruminants to HS

Exposure of ruminants to HS induces several responses at different levels (phenotypic and cellular) as shown in Figure 1.5. In the following sections, these responses will be covered using the available published research work in sheep in addition to studies carried out in cattle and goats.

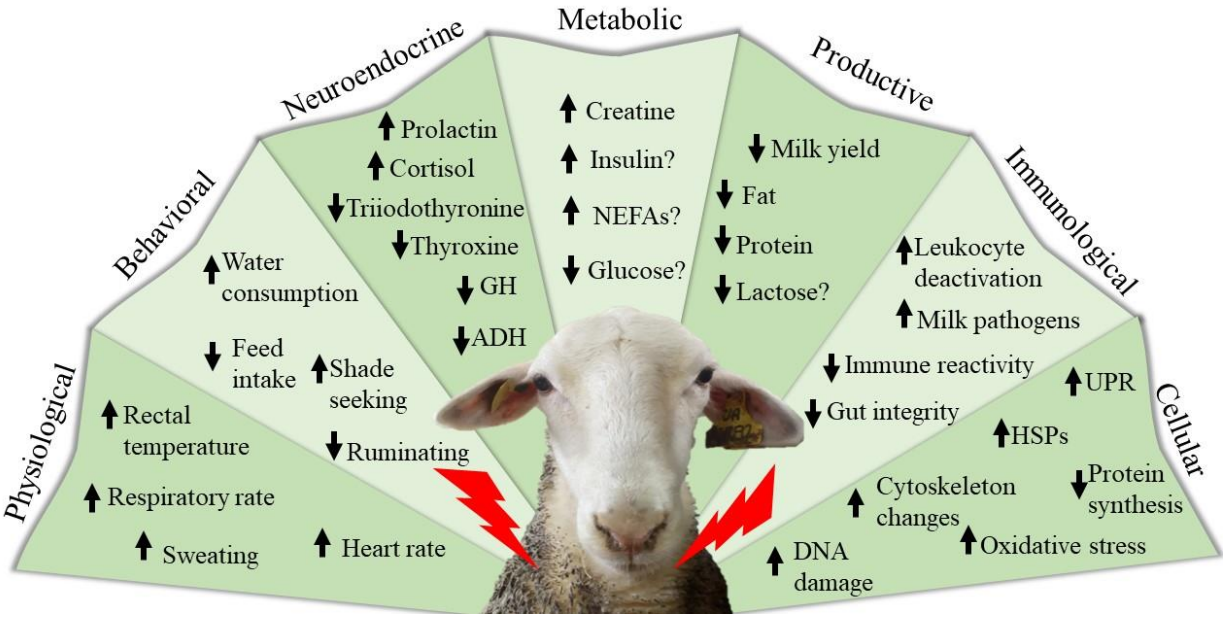


Figure 1.5. Heat stress responses in small ruminants

1.7.1 Phenotypic responses

1.7.1.1 Behavioral responses

When animals are exposed to HS conditions the major behavioral responses include the reduction of feed intake, an important source of heat production in ruminants (West, 2003), to prevent metabolic thermogenesis (Sejian et al., 2018; Pragna et al., 2018). Heat causes rostral cooling center of the hypothalamus to stimulate the medial satiety center and inhibit the lateral appetite center; the result is decreased feed intake (Albright and Alliston, 1971) and energy intake that coupled with increased maintenance costs during HS (7 to 25% increase, according to NRC, 2001). Consequently, heat-stressed animals suffer negative energy balance (NEBAL) as reported in lactating cows (Bernabucci et al., 2010). The decreased milk synthesis during HS conditions is partially explained by this reduction of feed intake (Baumgard and Rhoads, 2013).

In contrast, HS markedly increases water and ions losses of ruminants, and hence their requirements by acting as the primary vehicle for evaporative dissipation (sweating and panting) (Beed and Collier, 1986) and exerting direct cooling of the reticulum-rumen, which reduces core body temperature effectively (Garner et al., 2017). As described by Marai et al. (2007), sheep exposure to heat load induces a marked increases in water turnover and water intake.

Shade seeking behavior is an attempt of animal to ameliorate the direct effect of heat load (Sejian et al., 2018). It has been reported that access to shade reduces respiratory rate, panting score and DNA damage of dairy cows exposed to HS (De Abreu et al., 2020).

1.7.1.2 Endocrine responses

Endocrine system coordinates metabolic events during thermal stress (Beede and Collier 1986). A summary of the main endocrine responses to HS in cattle are presented in Table 1.5. Thyroid hormones, prolactin, GH, glucocorticoids, and mineralocorticoids are the main hormones implicated in the acclimatory response to heat (Bernabucci et al., 2010) and some of them will briefly explained in such a context. The reduced blood levels of triiodothyronine (T3) and thyroxine (T4) would allow for the adjustment of metabolic rates on favor of decreased energy utilization and heat production during heat load exposure (Kahl et al., 2015). Furthermore, the decline in thyroid hormones and GH have synergistic effect in reducing heat production (Yousef and Johnson, 1966).

In the case of the multifunctional hormone prolactin, its plasma increase has been reported in heat-stressed ewes (Hooley et al., 1979; Stephenson et al., 1980; Hill and Alliston, 1981), goats (Sano et al., 1985) and cows (Ronchi et al., 2001). Prolactin is involved in meeting the increased water and electrolyte demands of heat-stressed animals to maintain the extracellular fluid volume, and hence supporting heat dissipation (Alamer, 2011).

Plasma cortisol rises markedly (stimulus for the immune system) when cattle are acutely exposed to high environmental temperatures and decreases (potential immune suppression) during the chronic phase due to hypothalamic-pituitary-adrenal and sympathetic-adrenal-medullary axes activation (Silanikove 2000; Cantet et al., 2021), implying immune system disruptions that will be described later.

Table 1.5. Some endocrine responses during heat acclimation in cattle (Adapted from Bernabucci et al., 2010).

Tissue	Response		References
Adrenal	Aldosterone	Reduced	Collier et al. (1982a)
	Glucocorticoid	Reduced	Collier et al. (1982b), Ronchi et al. (2001)
	Epinephrine	Increased	Alvarez and Johnson (1973)
	Progesterone	Increased	Collier et al. (1982b), Ronchi et al. (2001)
Adipose	Leptin	Increased	Bernabucci et al. (2006)
Pituitary	Prolactin	Increased	Ronchi et al. (2001)
	Somatotropin	Reduced	McGuire et al. (1991)
Thyroid	Thyroxine	Reduced	Collier et al. (1982b), Nardone et al. (1997)
Placenta	Estrone sulphate	Reduced	Collier et al. (1982b)
Pancreas	Insulin	Increased	Wheelock et al. (2010)

1.7.1.3 Metabolic responses

The endocrine alterations in HS as mentioned above will have effects on carbohydrate, lipid, and protein metabolism. In this context, liver have pivotal role given that liver is the central metabolic junction further moderating and distributing nutrients to peripheral tissues for maintenance or productive functions such as muscle deposition or milk synthesis (Seal and Reynolds, 1993). Insulin is a potent regulator of both carbohydrate and lipid metabolism and may play an important role in mediating how HS regulates post-absorptive nutrient partitioning (Baumgard and Rhoads, 2013). In the case of glucose, insulin regulates its uptake into muscles and adipose tissue via insulin-stimulated translocation of glucose transporter type 4 (GLUT-4)

(Cheatham et al., 1996) and most probably is responsible for hypoglycemia (−21%) reported in heat-stressed dairy cows (Yue et al., 2020). On the contrary, no HS effects on blood glucose have been reported in dairy goats (Hamzaoui et al., 2013; Salama et al., 2014; Mehaba et al., 2019), dairy ewes (Mehaba et al., 2021), and meat ewes (Sano et al., 1983), the latter only exhibited lower (−15%) glucose metabolism.

As antilipolytic hormone, the stimulated insulin levels may explain the lack of increased NEFA levels in heat-stressed cows although they suffer NEBAL (Wheelock et al., 2010), implying that heat-stressed animals appear metabolically inflexible. The restriction of adipose tissue mobilization prevents glucose sparing mechanism for milk synthesis, but it is considered as a strategy to minimize metabolic heat production (Baumgard and Rhoads, 2013). The results vary in the case of small ruminants since no changes in NEFA were detected in goats (Mehaba et al., 2019) and sheep (Alhidary et al., 2012; Mehaba et al., 2021), while Sevi et al., (2001) reported higher plasma NEFAs values in dairy ewes exposed to solar radiation fed in the morning or afternoon during summer season.

Given that ruminants obtain little to no glucose directly from dietary digestion, gluconeogenesis is vital to supplying extra hepatic tissues with glucose through hepatic precursors such as propionate, amino acids, lactate, and glycerol (Rhoads et al., 2011). In the context of HS, protein catabolism of skeletal muscle is increased, which seems to fulfill the amino acids availability for hepatic gluconeogenesis and acute phase proteins (Baumgard and Rhoads, 2013; Abbas et al., 2020). In this sense, the increased levels of urea resulting from hepatic deamination of amino acids mobilized from skeletal muscle (Bernabucci et al., 2010), in plasma, milk or urine, was detected in heat-stressed dairy cows (Wheelock et al., 2010; Gao et al., 2017) yet not in goats (Hamzaoui et al., 2013) or ewes (Mehaba et al., 2021). In accordance with protein catabolism, increased levels of creatinine, an indicator of muscle degradation, was reported in dairy ewes (Mehaba et al., 2021), goats (Mehaba et al., 2019) and cows (Srikandakumar and Johnson 2004) under HS conditions.

The altered postabsorptive protein metabolism in response to HS is reflected in changes in the quantity of carcass lean tissue in different species (Close et al., 1971; Lu et al., 2007). In line with this notion, the reduced protein synthesizing machinery and RNA as well as DNA synthesis

capacity reported under environmental hyperthermia (Streffer, 1982) would partially explain the lower protein synthesis in mammary gland as described below.

1.7.1.4 Lactation performance

Depending on the severity, HS can exert detrimental effects on milk production in dairy animals leading to significant negative economic losses for dairy industry (Garner et al., 2020). The reduction of milk yield and worsen milk composition were generally related to feed intake reduction. However, Rhoads et al. (2009) and Wheelock et al. (2010) reported that only 35-50% of decrease in milk yield is explained by feed intake reduction in pair feeding experiments carried out with dairy cows. That is, HS may directly affect milk yield by specific mechanisms that are independent of reduced DMI (Rhoads et al., 2009). The implicated mechanisms could be related to port-absorptive metabolism, insulin sensitivity, nutrient partitioning, and cellular pathways responses addressed to cell survival (Rhoads et al., 2009; Wheelock et al., 2010). The negative effects of HS on milk fat and protein are well documented in dairy cows (Gao et al., 2017), goats (Hamzaoui et al., 2013; Salama et al., 2014), and ewes (Ramón et al., 2016; Mehaba et al., 2021).

1.7.1.5 Immune system

It has been reported that HS has negative impact on immune system via cell mediated and humoral immune responses, which results in more susceptible animals to diseases (Bagath et al., 2019, Dahl et al., 2020). Available results in dairy cows and goats showed that HS causes a reduction of immunoglobulins (IgG and IgA) in colostrum (Nardone et al., 1997), an impairment of blood polymorphonuclears cells functions as phagocytosis and oxidative burst (Lecchi et al., 2016), a decline of peripheral blood mononuclear cells reactivity (Lacetera et al., 2006), a decreased cytokine secretion of lymphocytes (Do Amaral et al., 2010), a delayed somatic cell recruitment after intramammary endotoxin challenge (Salama et al., 2020), and a downregulation of leukocyte transendothelial migration pathway in blood cells (Contreras-Jodar et al., 2018). Regarding sheep, exposure to solar radiation reduced *in vivo* cellular immune reactivity and had detrimental effect on milk hygienic quality of dairy ewes since it increases the number of pathogen microorganisms and polymorphonuclear neutrophil leukocyte counts (Sevi et al., 2001). The specific mechanisms underlying reduced cellular immune function in

sheep under HS remain undefined, especially cytokine profiles responsiveness (Sevi and Caroprese, 2012).

Consequences of high environmental temperature, particularly for the immune system and intestinal health of mammals, are a topic of rising interest given that HS directly alters jejunal tight junction proteins and recruit immune cells populations into the intestine, suggesting an impaired intestinal barrier in dairy cows (Koch et al., 2019). This is supported by Contreras-Jodar et al. (2019) who reported the presence of gut derived toxic compounds generated by gastrointestinal microbiota (hippurate, and phenylalanine derivative compounds) detected by urinary metabolomics analysis from dairy goats exposed to HS conditions.

Immune responses to HS seem to evolve in two phases: an early inflammatory phase and a late immunosuppressive phase (Biswas and Lopez-Collazo, 2009). The early phase is characterized by leukocyte activation, cytokine storm, and even a systemic inflammatory response, while in late phase the immunosuppression involves leukocyte deactivation and increased risk of secondary infection (Shalova et al., 2015). Table 1.6 summarizes the main phenotypic responses (e.g., thermo-physiologic, metabolic, endocrine, and productive) to HS conditions in sheep.

Table 1.6. Summary of main effects detected in sheep when exposed to heat stress conditions (HS = heat stress, CO = control).

Reference	Species and Breed	State	Treatments		Main effects			
			HS	CO	RT and RR	Intakes and BW	Milk yield and composition	Other
Stephenson et al. (1980) (Exp.1)	Sheep Merino ewes (n = 20)	Lactating	Climatic chamber (4d)	TN room (4d) 18-30°C/ RH 30-70 %	RT (+2%), RR (+194%)	No effect on DMI, water (+27%)	No effects	Blood indicators: Prolactin (+220%)
Sano et al. (1983)	Corriedale ewes (n=5)	Shorn	Climatic chamber (10 d) 30°C / 70% RH	Climatic chamber (10 d) 20°C / 70% RH	RT (+3%), RR (+500%)	No effect on BW	n/d	Blood indicators: NEFA and T4 (↓), glucose turnover rate (−15%)
Sevi et al. (2001)	Sheep Comisana ewes (n=40)	Late lactation	Summer season, unshaded (6wk) 18-38°C / 24-74% RH / THI >80	Summer season, shade (6wk) 21-32°C / 30-67% RH / THI < 75	Unshaded vs. shade RT (+4%), RR (↑)	n/d	No effects on milk yield and SCC. PMNLC (↑)	Blood indicators: NEFA (+15%), ALT (−27%), ALP (−18%). No effects on glucose
Al-Haidary (2004)	Sheep Naimey (n=8)	−	HS room (3 wk) 33-38.5 °C	TN room (3 wk) 24°C / 50% RH	RT (+1%), RR (+31%)	n/d	n/d	Packed cell volume (+7%), mean cell hemoglobin (−4%), no effect on T3, T4
Peana et al. (2007)	Sheep Sarda ewes (n = 10)	Lactating (5-6 mo)	Summer season (28d) THI (72-75)	Summer season (28d) THI (60-65)	n/d	DMI (−20%)	Yield (−20%) No effect on composition	n/d
Alhidary et al. (2012)	Sheep Merino wethers (n = 12)	9 mo old castrated	Climatic chamber (7d) 28-40°C / 47-57% RH / THI 75-93	Climatic chamber (7d) 19-26°C / 48-63% RH / THI 63-74	RT (+2%), RR (+179%)	DMI (−22%), BW (−5%), Water (+238%)	−	Creatine (−15%), no effects on glucose, total protein, cholesterol, NEFA, calcium, sodium
Wojtas et al. (2014)	Sheep Merino (n = 15)	Rams 12 mo old	Climatic chamber (7 d) 29-31°C / 48-51% RH / THI 77-79	Climatic chamber (7 d) 20 to 21°C / 74% RH / THI 69-70	No effects on RT, RR (+72%)	n/d	−	White blood cell count (−9%), K (−9%), Cl (+11%), Ca (+18%), no effect on cortisol
Mehaba et al. (2021)	Sheep Lacaune (n=8)	Lactating	Climatic chamber (21 d) 28-35°C / 45% RH / THI 75-83	TN room (21d) 15-20°C / 50% RH / THI 59-65	RT (+2%), RR (+214%)	DMI (−11%) BW(−18g/d) Water (+28%)	No effect on yield. Fat (−13%), protein (−16%), lactose (+7%)	Creatinine (+21%) No effects on blood glucose, NEFA or urea

ALP = alkaline phosphatase; ALT = alanine amino transferase; BW = body weight; DMI= dry matter intake; NEFA = non-esterified fatty acids; PMNLC = polymorphonuclear neutrophil leukocyte count; RH = relative humidity; RR = respiratory rate; RT = rectal temperature; SCC = somatic cell count; T3 = triiodothyronine; T4 = thyroxine; THI = temperature humidity index; TN = thermoneutral; ↑ = increase; ↓ = decrease.

1.7.2 Cellular responses

Cellular exposure to high temperatures alters its functions that in turn triggers cellular stress responses, which are part of normal physiology to either ensure the cells survival or alternatively to eliminate damaged or unwanted cells. Whether cells acquire a protective or destructive stress response is dependent on nature and persistence of stress itself, and also on cell type (Fulda et al., 2010).

According to Sonna et al. (2002) HS effects on cellular function could be comprised in seven points: 1) inhibition of DNA synthesis, transcription, RNA processing and translation; 2) inhibition of progression through the cell cycle; 3) denaturation and misaggregation of proteins; 4) increased degradation of proteins; 5) disruption of cytoskeleton; 6) alterations in metabolism that lead to a net reduction in cellular ATP; and, 7) changes in membrane permeability leading to an increase in intracellular Na^+, H^+, and Ca^{2+}. Faced with such a variety of anomalies in cellular functions, heat (as one stressor factor) induces different cellular responses depending on its intensity and persistence, that could lead acclimated state, a process largely controlled by the endocrine system (Collier et al., 2008).

Correspondingly, these cellular dysfunctions invoke a highly conserved cascade of protein activation and altered gene expression collectively known as the "heat shock response" (Lanks, 1986; Lindquist, 1986). As reviewed by Collier et al. (2008), these changes in gene expression patterns (reprogramed transcriptome) include: 1) activation of heat shock transcription factor 1 (HSF1); 2) increased expression of heat shock proteins (HSP) with decreased expression and synthesis of other proteins; 3) increased glucose and amino acid oxidation and reduced fatty acid metabolism; 4) endocrine system activation of the stress response; and, 5) immune system activation via extracellular secretion of HSP. Some of these responses will be detailed below.

1.7.2.1 Altered protein synthesis under HS

A small increase in temperature can cause protein unfolding, entanglement, and unspecific aggregation. In fact, many of the phenotypic effects of HS can be explained by the imbalance of protein homeostasis and its aggregation (Richter et al., 2010). Altering protein conformation results in an influx of misfolded or aggregated proteins to endoplasmic reticulum (ER) lumen

(Bouchama et al., 2017). The ER is a large membrane-enclosed cellular organelle, found in all eukaryotes, and it is the primary site of folding, maturation, and degradation of secreted and membrane-bound proteins, ensuring that only properly folded proteins are delivered. Further, it synthesizes lipids and sterols, and it is the main store of free calcium (Lin et al., 2008; Almanza et al., 2019). As nascent polypeptides enter the ER lumen, they are modified by N-linked glycans (composed of 2 N-acetylglucosamine, 9 mannose, and 3 glucose molecules) to be folded appropriately into secondary and tertiary structures. These processes are assisted and monitored by ER chaperones and folding enzymes (Ma et al., 2004).

The perturbation of ER homeostasis is termed as ER stress and occurs by the accumulation of unfolded/misfolded proteins, and when ER calcium is depleted (Liu and Green 2019). The ER responds by activating adaptive intracellular signal transduction pathways collectively known as the unfolded protein response (UPR), which is used to align ER functional capacity with demand (Cnop et al., 2012; Walter and Ron, 2011). As consequence of HS, diverse cellular stresses, such as disruption of calcium homeostasis, redox imbalance and protein folding defects cause defective proteins (misfolded and unfolded) accretion in the ER lumen, which triggers UPR (Wu et al., 2020). The UPR regulation involve transmembrane ER-resident proteins, including inositol-requiring protein 1 (IRE1), PKR-like endoplasmic reticulum kinase (PERK), and activating transcription factor 6 (ATF6). These proteins bear domains protruding into the ER lumen, which act as sensors of ER stress (Lin et al., 2008) and it was reported that HS activates them to protect cell against the imposed ER stress (Homma and Fujii, 2016). In non-stressed conditions, ER stress sensors maintain inactivated by contact with the binding immunoglobulin protein or glucose regulated protein 78 (BiP/GRP78) chaperon and it is during ER stress when BiP is displaced to interact with misfolded luminal proteins, leading to IRE1, PERK and ATF6 activation as shown in Figure 1.6 (Flamment et al., 2012). The subsequent steps (for review see Hetz et al., 2011; Flamment et al., 2012) lead in a transitory attenuation of protein synthesis and a transcriptional activation of genes to increase the protein-folding capacity of the ER (Heldens et al., 2011).

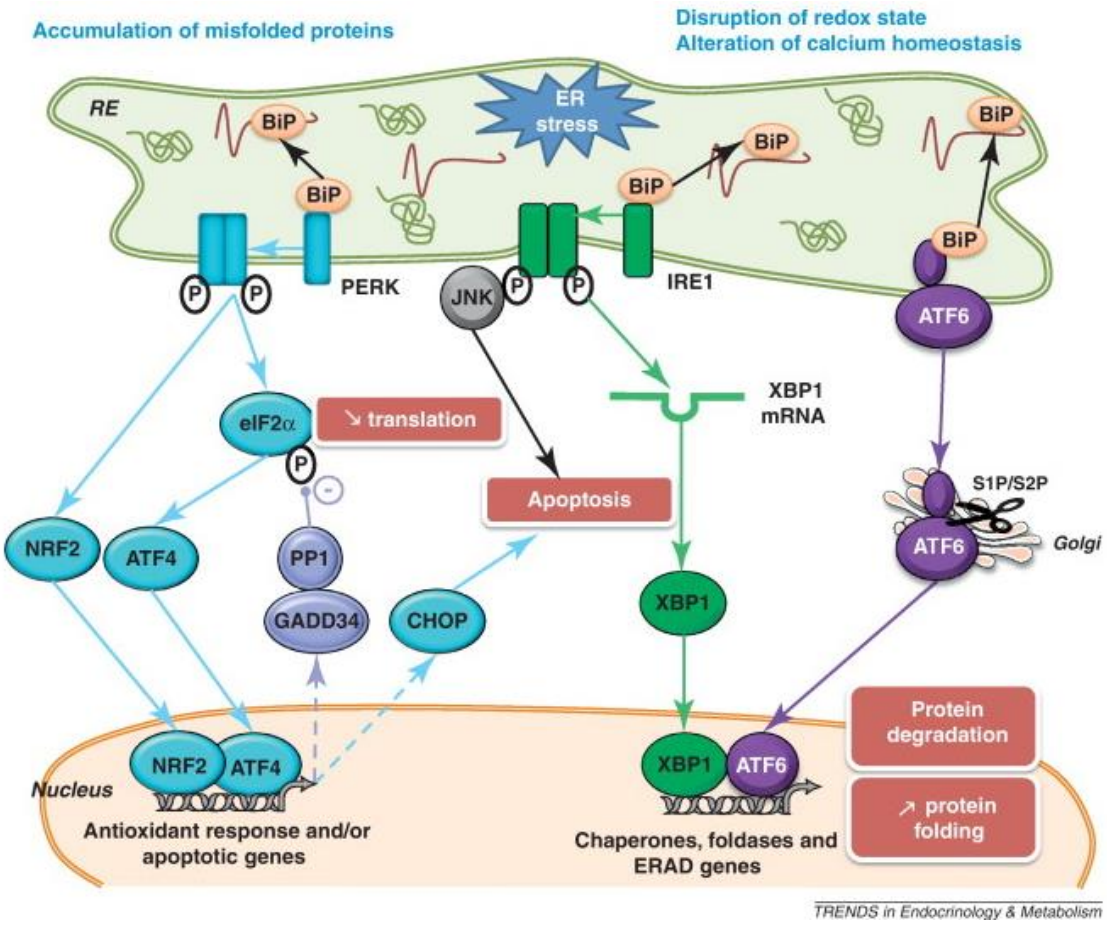


Figure 1.6. Unfolded protein response activation and cascades of events in the endoplasmic reticulum (Flamment et al., 2012).

Although protein synthesis machinery is disturbed under HS, this does not apply to HSP synthesis (Belhadj et al., 2016). These HSP are divided into different families according to their molecular weights, where the most widely studied have molecular weights of roughly 27, 70, and 90 kDa, referred as HSP27, HSP70, and HSP90, respectively (Guerriero and Raynes, 1990). These proteins act as molecular chaperones to avoid inappropriate protein aggregation and shipment regulation of immature proteins to the target organelles for packaging, degradation, or repair (Kiang and Tsokos, 1998). Thus, HSP have a major role in heat tolerance and protection of heat-exposed cells. Among these, HSP70 stands out in cytoprotective effects and provide cells with time to repair the damage induced by a variety of stresses by interfering with programmed cell death signaling pathways (Volloch and Rits, 1999). Consequently, this HSP70

class is known for its potential role in thermotolerance and widely considered as cellular thermometers where its expression/accumulation rate in cells has been related with the development of thermotolerance (Hassan et al., 2019). The upregulation of both genes encoding HSP70 and HSP90 from blood cells were reported in goats (Dangi et al., 2012) and cows (Liu et al., 2020). The upregulation of HSP70 was also detected in sheep (Romero et al., 2013) and the mammary gland of cows (Yue et al., 2020) that were exposed to HS.

1.7.2.2 Oxidative stress

Production of reactive oxygen species (ROS) is a result of normal cellular metabolism, hence at low to moderate concentrations, they are part of physiological cell processes, but at high levels they cause adverse modifications to cell components, such as lipids, proteins, and DNA (Valko et al., 2006). The imbalance between oxidant/antioxidant in favor of oxidants is termed "oxidative stress" (Birben et al., 2012). In different mammalian cells oxidative stress causes free-thiol oxidation and appearance of oxidized proteins, cytoskeleton disassembly, depletion of pyridine nucleotide and ATP pools, increased plasma-membrane peroxidation and permeability, release of cytosolic components, free Ca increase, and breaking of DNA strands (Dalle-Donne et al., 2001). Several studies reported that exposure to high environmental conditions increases ROS production and induces oxidative stress in dairy cows (Bernabucci et al., 2002a; Tanaka et al., 2007; Li et al., 2021).

Heat stress causes oxidative stress by inducing mitochondrial dysfunction characterized by increasing the permeability of the mitochondrial inner membrane and impairing oxidative phosphorylation (Willis et al., 2000; Guo et al., 2021). Hence, mitochondria are thermosensitive and one detrimental effect of ROS is the inactivation of the respiratory chain (mitochondrial basic function) via the oxidation of complexes I, II, IV and V (Ozawa et al., 1987; England et al., 2004). This implies that electron flow slows down and ATP synthesis declines (Zhao et al., 2006), resulting in downregulation of cellular energy production. Heat induced mitochondrial damage may be responsible of cell inability to meet increased energy requirements of heat-stressed animals (Belhadj et al., 2016).

In many cell types, including hepatocytes, oxidative stress produces a severe disruption of the actin cytoskeleton characterized by fragmentation and patching of F-actin (Dalle-Donne et

al., 2001). This fact could partially explain why cytoskeleton rearrangement is one of the major HS damages reported (Welch and Suhan, 1985; Richter et al., 2010; Toivola et al., 2010).

1.8 Heat tolerance

Theoretically, a heat tolerant animal maintains homeothermy when exposed to heat load. From a livestock point of view, a tolerant animal is that one able to sustain productive and reproductive levels under such conditions (Carabaño et al., 2019). The selection only for milk production have resulted in reducing HS tolerance in dairy cows (Ravagnolo and Misztal, 2000) and dairy sheep (Finocchiaro et al., 2005), implying that selection indices including milk production and heat tolerance might be used to overcome this antagonistic relationship (Carabaño et al., 2019).

Amongst species, goats and sheep are considered more heat tolerant in comparison with cattle (Silanikove 2000), the latter having higher metabolic rate and poorly developed water retention mechanism in the kidney and gut (Bernabucci et al., 2010).

In sheep, breed differences are demonstrated by the reported higher estrus rate, lower respiratory rate, and heart rate in Turpan Black sheep (heat resistant) compared with Kazakh sheep (heat sensitive) under summer season (Haire et al., 2022). Similarly, Romero et al. (2013) reported that Pelibuey sheep experience more effective thermoregulation (increase of rectal temperature to lesser extent) in response to HS, and higher survival of blood mononuclear cells with a markedly increment of HSP-70 concentration than shorn Suffolk sheep after HS exposure. As discussed above, animal HS response widely varies, reaching up to individual-individual differences. Currently, genetic evaluations to select heat tolerant animals are based on production decline analysis under high heat loads, supporting there is individual genetic variability in the response to HS, which makes feasible the selection for heat tolerance (Ravagnolo and Misztal, 2000; Carabaño et al., 2019). There are different sets of candidate genes highly associated with thermotolerance in small ruminants (Figure 1.7), including heat shock protein genes.

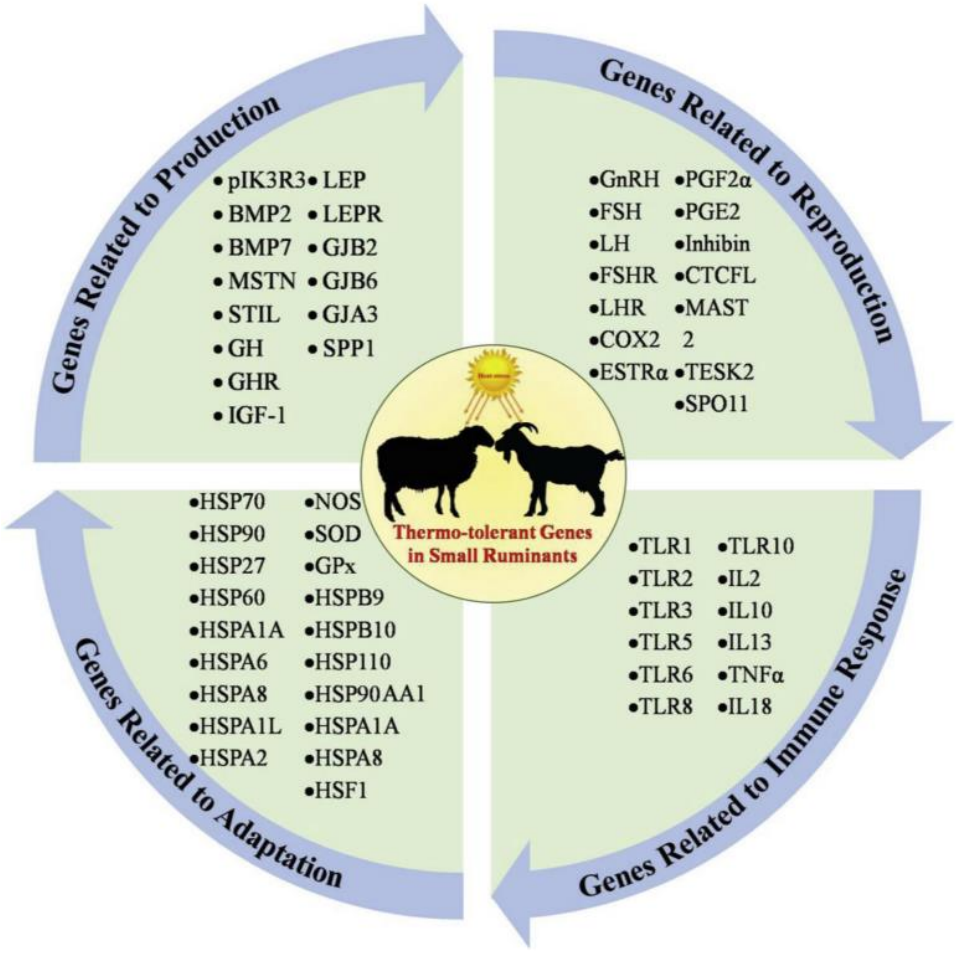


Figure 1.7. Genes associated with thermotolerance in small ruminants (Sejian et al., 2019)

Whilst the analysis of phenotypic responses under HS conditions have contributed to identify heat tolerant animals as mentioned, "Omics" tools as transcriptomics would help explain the involved mechanisms in HS tolerance. Transcriptomics is defined as the study of the complete set of RNA molecules expressed in one cell or a population of cells and quantify the changing expression levels of transcripts for a specific developmental stage or physiological condition (Wang et al., 2009). Also, the type and number of genes transcribed is dependent on the cell-type and its environment (Srivastava et al., 2019), implying a dynamic nature of transcriptome since it captures a snapshot in time of the total transcripts present in a cell, therefore is a good representative of cellular state (Lowe et al., 2017) under HS.

The research based on transcriptome have yielded insights into the molecular basis of phenotype in response to HS using different tissues, including blood (Lacetera et al., 2006; Do Amaral et al., 2010; Liu et al., 2020), mammary gland (Gao et al., 2019; Salama et al., 2019; Yue et al., 2020), and liver (Shahzad et al., 2015; Koch et al., 2016) in dairy cows.

Heat stress response is an intriguing and dynamic process that stimulate gene expression in various tissues including the liver, the central metabolic junction in nutrients partitioning as mentioned earlier. In Hu sheep, liver transcriptomics revealed differentially expressed genes related to metabolic processes, regulation of biosynthetic processes, and glucocorticoids when comparing summer and autumn seasons (Li et al., 2019). When contrasting August (THI > 26) and December (THI < 11) the liver transcriptome profiling was associated with stress response, immune reaction, and fat metabolism in the same breed (Lu et al., 2019). As far as we know, no data are available on liver transcriptomics in dairy ewes under HS conditions.

CHAPTER 2

Objectives

CHAPTER 2

Objectives

Addressing the Earth's global warming phenomenon in the context of dairy sheep, the broad goal of this thesis was to explore of the effects of an immediate alleviation management practice (i.e., shearing strategy), as well as a mid-term approach aiming to understand the differences between heat sensitive and tolerant phenotypes of dairy ewes for future genetic improvement.

Therefore, the specific objectives of this Thesis were:

- To evaluate the effects of 3 shearing strategies (no shearing, shearing before breeding, and shearing during pregnancy) on the lactational traits (colostrum quality, milk yield and composition, cheese-yielding traits), metabolic status (e.g., body reserves, bodyweight, blood indicators), and offspring performances (e.g., lamb bodyweight) in Lacaune and Manchega ewes pregnant during summer and lactating thereafter.
- To assess the performance (e.g., feed and water intakes, milk yield and composition) and metabolic (e.g., blood indicators, glucose tolerance test kinetics) responses of lactating Manchega dairy ewes classified with divergent heat tolerance phenotypes (sensitive and tolerant based on respiratory rate/rectal temperature change ratio) exposed to thermoneutral and heat stress conditions.
- To carry out an integrated liver transcriptome and pathway analyses in sensitive and tolerant phenotypes of Manchega dairy ewes above evaluated under thermoneutral and heat stress conditions.

CHAPTER 3

When to shear dairy ewes: before breeding, during pregnancy or let them unshorn?

CHAPTER 3

When to shear dairy ewes: before breeding, during pregnancy or let them unshorn?[1]

3.1 ABSTRACT

Aiming to evaluate whether shearing strategy affects the gestational and lactational performances of dairy ewes, 73 pregnant ewes (MN, Manchega, n = 43; LC, Lacaune, n = 30) were divided in 3 balanced groups to which the experimental treatments were randomly allocated. Treatments were: i) control, ewes maintained unshorn (CO, n = 24), ii) ewes shorn before breeding (SBB, n = 23), and iii) ewes shorn at d 100 of pregnancy (S100, n = 26). Ewes suckled their lambs and were machine-milked until d 180. Fleece features and respiratory rate (RR) of the ewes were assessed under summer at d 100 of pregnancy. Lamb and ewe body weight (BW) were recorded throughout the experiment as well as body condition score (BCS) of the ewes. Blood and colostrum were sampled at lambing. Milk was assessed during suckling and sampled for composition. Machine-milk was recorded daily and sampled fortnightly. Batch-milk samples were taken for cheese-yielding traits at late lactation by treatment. Fleece extension and wool weight were 13% and 45% greater, in MN than LC ewes, respectively. Ewes' RR at different ambient temperatures did not vary by breed and S100 ewes had 37% lower RR than SBB and CO ewes at 28ºC. At lambing, SBB and S100 ewes had, on average, 86% greater glycemia than CO in MN ewes, but it did not vary in LC ewes. No differences among treatments were detected for plasma insulin, β-hydroxybutyrate (BHB) and non-esterified fatty acids (NEFA) in both breeds. S100 ewes had greater BCS than CO and SBB in both breeds. At lambing and during lactation, CO ewes were heavier than SBB. Shearing treatment did not affect neither colostrum composition nor milk yield throughout lactation in any breed. However, S100 ewes tended to yield 28% more milk during milking than CO in the LC ewes. Regarding milk composition during suckling, protein and casein contents increased by 6% when S100 and CO treatments were compared in MN ewes, whereas total solids (8%) and fat (8%) milk contents increased in S100 compared to SBB, in LC ewes. Lamb's growth during suckling did not differ by treatment in both breeds. No effects on milk composition during milking were detected by shearing treatment. Finally, no effects on cheese-extract and on milk coagulation properties were detected by treatment, except in the case of rennet coagulation time, with opposite effects by breed. Thus, the S100 milk needed 9% more time to form the curd than CO milk, for MN ewes, and on the contrary, S100 milk coagulated faster (8%) than CO and SBB milks in LC ewes. In conclusion, shearing pregnant ewes during summer may be a recommendable management practice, without detrimental effects on the lambing and lactational performances of dairy ewes.

[1]This article was submitted to *Animal* journal as: S. González-Luna, L. Cordón, A. A. K. Salama, X. Such, E. Albanell, A. Contreras-Jodar, J. de Lucas-Tron, and G. Caja. When to shear dairy ewes: before breeding, during pregnancy or let them unshorn? (ANIMAL-22-30328).

3.2 INTRODUCTION

Shearing is a husbandry practice which is economically important for the revenue of fine-wool sheep farms. Nevertheless, this income is negligible in dairy sheep (< 0.8%; Milán et al., 2014). Timing and frequency of shearing varies widely according to climatic conditions and production systems but, in dairy ewes, it is mainly done during the dry period and addressed either to alleviate heat-stress during summer (i.e., open ewes in spring) or to enhance ewe's fitness before parturition (i.e., pregnant ewes in winter). Dairy ewes can also be shorn in mid-lactation during mild-winter for milk hygiene and milking easiness, without negative effects on milk yield and composition (Elhadi et al., 2019).

Pregnancy shearing was first studied by Rutter et al. (1971) in Greyface and Cheviot ewes at winter housing, who reported that shearing at mid-pregnancy increased birthweight and reduced lamb mortality by improving the mothering behavior of the ewes. These effects were later confirmed by Rutter et al. (1972), although no differences in food intake were detected. Symonds et al. (1986) reported increased fat catabolism in Blue-faced Leicester × Swaledale ewes shorn at d –56 of pregnancy, without changes on NEFA, BHB, carbohydrate or protein metabolism, but with lower insulin secretion, which resulted in numerically greater glycemia during late pregnancy. Shorn ewes seem to be better adapted to late pregnancy than the unshorn ones.

Pregnancy shearing was proposed for improving the survival of the lambs to respond to the rise of ewe's prolificacy (Kenyon et al., 2003). Nevertheless, results are in many cases inconsistent and dependent on the potential of the ewes (i.e., breed giving lambs of low birthweight) and on resources available (i.e., adequate body reserves or nutrition), as reviewed by Kenyon et al. (2003). Pregnancy implies a complex regulation of nutrient partitioning to support the development of conceptus and mammary gland which have metabolic priorities under homeorhetic and homeostatic controls (Bauman and Currie, 1980). Moreover, lamb birthweight and inhibition of insulin secretion during glucose tolerance tests associated to shearing pregnancy are more pronounced in twin-bearing ewes (Revell et al., 2000).

Regarding lactation performances, results of pregnancy shearing are controversial, varying according to timing (i.e., days to lamb), purpose (i.e., meat, wool or dairy), season (i.e., summer or winter) and farming conditions (i.e., housed or grazing). Knight et al. (1993) reported no

effect of shearing at late-pregnancy (d 115, on average) on milk yield of Dorset ewes that were machine-milked from parturition, although greater milk contents were observed. On the contrary, Cam and Kuran (2004) and Sphor et al. (2011) reported greater milk yield, but no changes on milk composition, when Karakaya and Polwarth ewes shorn at d 100 or d 53 of pregnancy, respectively, were compared to unshorn ewes during suckling. It should be stressed that both breeds are meat sheep and that milk samples were obtained by milking after 24-h lamb separation (Karakaya) or with the help of oxytocin (Polwarth), which more likely altered milk yield and composition. An early consequence of pregnancy shearing, which could support the greater survival and weaning weight of the lambs, may be colostrum quality, although Banchero et al. (2010) did not found differences between shorn and unshorn ewes.

On the other hand, when considering dairy sheep, García-Rodríguez et al. (2012) did not detect effects on milk yield and composition of Latxa dairy ewes shorn at late-pregnancy (d 110, on average), that were machine-milked after the weaning of the lambs under winter and housing conditions. The authors reported increases on twin-lamb birthweight (0.56 kg/lamb) and feed intake (15%) in the shorn ewes. Interestingly, Leibovich et al. (2011) compared the effects of pregnancy shearing (d –30) in Assaf dairy ewes that were machine-milked from lambing, under summer housing with or without fan-cooling. They reported increases in intake (8%), milk yield (7%) and milk composition (fat, 11%; protein, 3%; energy corrected milk, 10%) only in the case of shearing associated to cooling. Moreover, lamb birthweight increased by the joint effect of shearing and cooling (0.40 kg/lamb).

Given that sheep milk is mainly devoted for cheese production (Pulina et al., 2018), the balance among milk yield and milk composition effects on the curdling properties of milk are key for the cheese industry. To our knowledge, no study has investigated the effect of pregnancy shearing on milk coagulation traits.

Our hypothesis was that pregnancy shearing will increase lamb birthweight and milk yield, increasing colostrum and milk yield or composition, which may affect the coagulation traits of the milk of dairy ewes. To test our hypothesis, different shearing times related to the reproductive cycle of the ewes were compared: i) unshorn (as control), ii) shearing in late-spring before breeding (as done traditionally), or iii) shorn at the last-third of pregnancy (d 100). Effects of shearing strategies were assessed on the dairy performances and cheese-yielding traits of the

milk produced during the subsequent lactation of 2 breeds of dairy ewes differing in milk yield and milk composition.

3.3 MATERIALS AND METHODS

The experimental procedures and management practices reported in the present study were in accordance with the Ethics Committee on Animal and Human Experimentation of the Universitat Autònoma de Barcelona (UAB), the Spanish Royal Decree 53/2013 on the protection of animals used for experimental purposes, and the codes of recommendations for the welfare of dairy sheep of the Ministry of Agriculture, Alimentation and Environment of Spain (MAPA, 2007).

3.3.1 Animals, Feeding and Management Conditions

The research was carried out in the experimental farm of the Servei de Granges i Camps Experimentals (SGCE) of the Universitat Autònoma de Barcelona (UAB) in Bellaterra (Barcelona, ES). A total of 73 multiparous ewes of 2 dairy breeds (Manchega, **MN**, n = 43; Lacaune, **LC**, n = 30) managed as a unique flock, were used. The ewes had similar frame and BCS (2.68 ± 0.07 and 2.51 ± 0.05 points), but slightly differ on age (4.4 ± 0.3 and 3.8 ± 0.3 yr) and weight (73.0 ± 1.3 and 76.5 ± 1.8 kg BW). All ewes wore plastic ear tags (Allflex Europe, Vitré, FR) and ceramic rumen mini-boluses (20 g, half-duplex technology; Datamars, Bedano, CH) for visual and electronic identification, respectively.

Ewes grazed 6-h daily (1000 to 1600) on cultivated Italian ryegrass prairies, during winter and spring, and on natural pastures during autumn. Grazing was substituted by green chopped sorghum forage, fed ad libitum in the shelter, during summer. Ewes were sheltered on straw bedded pens after grazing and complemented with alfalfa hay ad libitum and concentrate according to requirements (INRAtion v.4.07 software; Educagri éditions, Dijon, FR). Corn whole grain (0.2 to 0.8 kg/d, as fed) and a farm-produced concentrate (0.4 to 1.0 kg/d, as fed; ingredients: soybean hulls, 60%; barley grain, 10%; oat grain, 10%; gluten feed, 10%; soybean oil, 4%; di-Ca phosphate, 2.5%; sugar cane molasses, 2%; Vitafac ovino-0.3% (DSM Nutritional Products, Madrid, ES), 1%; sodium chloride, 0.5%; as fed), were offered in 2 portions in the feeders of the pens (dry ewes) or in the milking parlor (suckling and milking

ewes) to meet their requirements. All ewes had free access to water and salt-micromineral blocks (Multi-Block; Agrària Comarcal del Vallès, Llerona, ES).

Breeding was done by rams, after ram-effect (i.e., rams isolated in contiguous pens for 10-d) in late-spring (mid-May and June), and lambing occurred in Autumn. After lambing, ewes and lambs were secluded in lambing pens for 5-d to reinforce bonding. Thereafter, the ewes and lambs joined the flock and suckled their lambs during the sheltering time (i.e., evening and night) until d 28 (abrupt weaning). Machine-milking was performed twice-daily (0700 and 1700) in a double-12 stall parallel milking parlor (Amarre Azul I; DeLaval Equipos, Alcobendas, ES) with a high milk pipeline, 12 milking clusters (SG-TF100 DeLaval, Tumba, SE) with milk flow and recording units (MM25-SG, DeLaval), and set to 40 kPa, 120 pulses/min and 50% pulsation ratio. The milking routine included manual cluster attachment, machine milking and automatic cluster detachment (milk flow rate <0.1 L/min or milking time >3 min). Individual teat dipping with an iodine solution (P3-ioshield; Ecolab Hispano-Portuguesa, Barcelona, ES) was done at the end of milking. Suckling ewes passed every day through the milking parlor to be fed with concentrate and to remove the milk surplus in the udder and for adapting to machine-milking routine.

3.3.2 Experimental Treatments

Ewes were blocked in 3 balanced groups by breed, age, and milk yield from the previous lactation, to which the treatments were randomly allocated. Treatments consisted of: i) control unshorn (CO), ii) shorn 15-d before breeding (SBB), and iii) shorn at d 100 of pregnancy (S100). The CO ewes were not shorn from the previous year and consisted of 24 ewes (MN, n = 14; LC, n = 10). The SBB group was shorn 15-d before introducing the rams (mid-May), and consisted of 23 ewes (MN, n = 13; LC, n = 10). The S100 group was shorn at d 100 of pregnancy (mid-August) and consisted of 26 ewes (MN, n = 16; LC, n = 10). Treatments are summarized in Figure 3.1. All groups were managed in a unique flock.

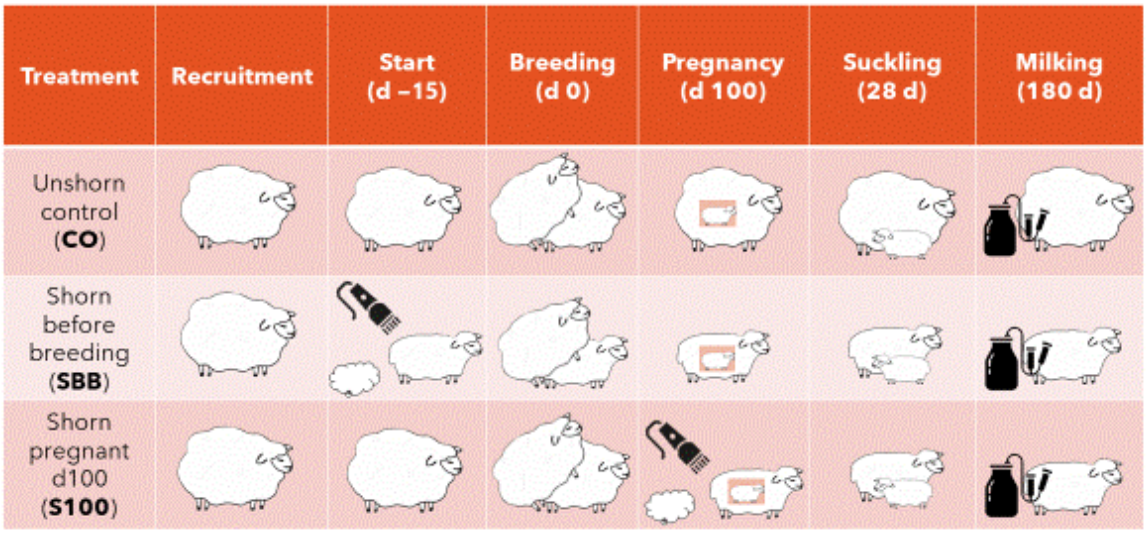


Figure 3.1. Shearing treatments in dairy ewes: left unshorn, shorn before breeding, or shorn at d 100 of pregnancy.

3.3.3 Measurements, Sampling and Analyses

Fleece Extension and Wool Weight. The fleece extension of all ewes was individually scored at d 100 of pregnancy using a three-point scale (score: 1, bare; 2, medium, 3; woolly; accuracy, 0.5 points) according to Elhadi et al. (2019). Shearing of SBB and S100 ewes was done by the same professional shearing team using the un-tied Bowen's technique and machine-shears (Evo-Heiniger 3 speeds machine with flexible drive-200 cm and Icon handpiece with Quasar shearing comb-95 mm wide; Heiniger, Herzogenbuchsee, SW). After shearing, the wool of each ewe was weighed using a digital scale (AND FV-60K; A&D Company, Tokyo, JP; accuracy, 0.02 kg) and recorded.

Respiratory Rate. With the aim of assessing the short-term effects of removing the fleece in the pregnant ewes during the summer conditions of our experiment, the measurement of the respiration rate (**RR**) was done by one trained operator at 3 different dates after S100 (d 107, 114 and 121 of pregnancy). Groups of 15 ewes per treatment, were randomly chosen for measurements among those that were in resting conditions (i.e., lying down after eating): Flank movements associated to breathing were counted by sight for 15 s and later expressed ×4 as RR (breaths/min or bpm). At each measurement, the ambient temperature was also recorded (range,

20 to 28°C) using an aerial probe suspended in the middle of the pen (STC 2020; SERTIC, Lleida, ES).

Body Reserves. Body weight (BW) of the ewes was measured with an electronic scale (Tru-test AG500; Auckland, NZ; accuracy, 0.2 kg) at different dates pre- (d –43, –33, –27, –21, –14 and –4) and post-lambing (d 0, 10, 36 and 68). The body condition score (BCS) of the ewes was also measured at the same time using the 0 to 5 points score (accuracy, 0.25 points) described by Russel et al. (1969). Lambs were weighed at birth, d 15, 21 and 28 (weaning) using a portable electronic scale (AND FV-60K; A&D Company).

Blood Measurements. Ewe's blood samples were taken from the jugular vein using 10 mL vacutainer tubes with sodium heparin 170 IU (BD; Belliver Industrial Estate, Plymouth, UK) as soon as possible after lambing. Plasma was obtained by blood centrifugation for 15 min at 1500 ×g and 4°C, transferred to 1.5 mL Eppendorf tubes and stored at −20°C until analysis. Concentrations of glucose, NEFA and BHB were determined from plasma using an Olympus AU480 analyzer (Olympus Europa, Hamburg, DE) with the specific Reagent System of Olympus (OSR; Beckman Coulter, Krefeld, DE) in the SBCV (Servei de Bioquímica Clínica Veterinària) of the UAB. The respective analytical methods and reagents used were: glucose by the hexokinase method (OSR6121) and read at 340 nm, NEFA by enzymatic colorimetry [ACS-ACOD-MEHA; acyl-CoA synthetase, acyl-CoA oxidase, 3-methyl-N-ethyl-N(βhydroxy-ethyl) aniline] using NEFA HR reagents (Fujifilm Wako Chemicals, Neuss, DE) and read at 410 nm. The BHB was determined by the kinetic enzymatic method using the Ranbut kit (Randox Laboratories, Crumlin, UK) and read at 340 nm. Plasma samples were also analyzed for insulin by ELISA sandwich type (Ovine Insulin; Mercodia, Uppsala, SE) and the stopped plates were read at 450 nm in an automatic reader (iEMS Reader MF V.2.9-0; Labsystems España, Barcelona, ES). Detection limit, intra- and interassay coefficients of variation were 0.025 ng/mL, 3.7% and 6.5%, respectively.

Colostrum. Colostrum samples were taken as soon as possible after lambing by hand-milking. Both udder sides were milked, mixed and 100 mL stored at −20°C until analysis. One sample of 50 mL was thaw at room temperature and conditioned to 40ºC previously to Near Infrared Analysis (NIRA) using a NIRA spectrometer (Foss Electric, Nordersted, DE) for content of total solids, fat, total protein (N × 6.38), true protein and casein. Calibrations were

performed using data obtained by conventional methods including total solids (oven at 103ºC), fat (Gerber method) and total protein (Kjeldahl method) as indicated by Albanell et al. (1999). For colostrum density values, a total of 10 ml of colostrum were put in a measuring cylinder (10 ± 0.1 mL) and weighed in a digital scale (Sartorius CP64, Gottingen, DE; accuracy, 0.1 mg) at 20°C. Density was calculated by the ratio between mass and volume. The remaining 50 mL of colostrum samples were thaw at 4ºC and centrifuged at 2,000 × g for 15 min at 4°C (Hettich Zentrifugen, D-78632 Tuttlingen, DE). The supernatant and bottom fractions were discarded, and the obtained skim milk preserved in 1.5 mL Eppendorf containers that were frozen at –20ºC until insulin (Ovine insulin, Mercodia, Uppsala, SE) and IgG (Calokit Ovino, ZeuLAB, Zaragoza, ES) analysis using ELISA Sandwich kits. Detection limit, intra- and interassay coefficients of variation for the IgG were 0.0051 mg/mL, 2.6% and 8.0%, respectively.

Milk Yield and Composition during Suckling. Milk yield during suckling was individually estimated at d 5, 14 and 28 of lactation using the double milking-oxytocin method (Doney et al., 1979) with a 4-h interval. With this aim, lambs and ewes were separated in the morning (0800) and the ewes moved to the milking parlor where they were injected oxytocin (2 IU/ewe; Facilpart, Laboratorios Syva, León, ES) into the jugular vein and machine-milked. The ewes returned to the pens after milking where they were fed, but remained isolated from their lambs. After 4-h the oxytocin injection and milking were repeated and the milk was individually collected, weighed in a portable electronic scale (AND FV-60K). Daily milk secretion was expressed (×6) as 24-h milk yield. Milk samples were taken (100 mL), preserved with an antimicrobial tablet (Bronopol, Broad Spectrum Micro-tabs II, D&F Control Systems, San Ramon, CA) and stored at 4°C until analyses. Gently mixed and conditioned milk samples were analyzed by NIRA, as previously indicated for colostrum.

Milk Yield and Composition during Machine Milking. Milk yield of individual ewes was recorded at each milking from d 29 to 180 of lactation by using the automatic milk-flow and milk-recording units (MM25SG, DeLaval) of the milking parlor. Data were uploaded at each milking by using the AlPro software 7.2 (DeLaval) and weekly reviewed for outsider values as indicated by Nieddu and Caja (2017). Representative milk samples (100 mL) of each ewe were taken at each milking at d 35, 49, 63 and 77 for composition analyses. Daily milk samples were composited (60:40) according to the daily milking interval (14- and 10-h, respectively),

preserved with an antimicrobial tablet (Bronopol) and stored at 4°C until analysis. Milk samples were analyzed using NIRA for fat, crude protein, true protein, casein and total solid contents according to Albanell et al. (1999).

Milk Coagulation Traits. Composited milk samples per ewe (100 mL) were collected at d 160 of lactation for assessing the milk coagulation properties and major composition analysis. Individual milk samples of the ewes of each experimental group were mixed, obtaining representative batches of the milk by shearing treatment and by breed. Milk batches were stored at 4ºC during the night and analyzed for determination of the coagulation and cheese-making properties on the following-day.

Coagulation variables were assessed in an Optigraph device (Ysebaert, Frepillon, FR), consisting of 10 wells (10 mL each) operating simultaneously and interfaced with a computer. Samples were previously conditioned at 34ºC for 15 min in a water bath. Coagulation temperature was set at 34ºC and the coagulation test lasted for 60 min. Coagulation properties of milk samples (RCT, rennet coagulation time; F45, firmness at 45 min) were determined in quadruplicate after addition of rennet enzyme (43 μL). Diluted rennet enzyme (1:10) was prepared by mixing 1 mL of calf rennet, containing 780 mg/L of chymosin (Larbus, Madrid, ES), with 9 mL of distillated water. Fresh diluted rennet enzyme was prepared and kept at 4ºC until use. The remaining individual milk samples (100 mL/ewe) were analyzed for fat, total protein, lactose, and total solids (Milkoscan FT2; Foss, Hillerød, DK) in the Dairy Herd Improvement Laboratory of Catalonia (ALLIC, Cabrils, Barcelona, ES) and the protein/fat ratio, fat-protein cheese-extract and the cheese yield (according to Van Slyke equation; Mullan, 2008) were calculated.

3.3.4 Statistical Analyses

Data analyses were performed using SAS v. 9.4 (SAS Inst. Inc.; Cary, North Carolina, USA) according to the nature of variables. Fleece extension was analyzed using the CATMOD procedure and wool weight was compared between breeds using the Student's t-test. Respiratory rate was analyzed by the MIXED procedure for repeated measurements of SAS using a model that contained the fixed effects of the breed, the shearing treatment, the ambient temperature, the random effect of the animal and the interaction of shearing treatment × temperature.

Considering the genetic and phenotypic differences between breeds (fleece, BW, BCS, milk yield and composition) the rest of the data of both breeds were analyzed separately. Thus, blood indicators of the ewes, colostrum composition, lamb birthweight and average daily gain during suckling, were analyzed using the MIXED procedure of SAS containing the fixed effects of shearing treatment, litter size and the interactions of litter size × shearing treatment. On the other hand, data of lamb weight, milk yield and milk composition, and ewe BW and BCS, were analyzed using the MIXED procedure for repeated measurements of SAS containing the fixed effects of the shearing treatment, the litter size, the recording (or sampling) time, the random effects of the animal and the interaction of litter size × shearing treatment. For ewe BW and BCS the interaction of shearing treatment × time recording was also included. Pearson correlation coefficients (r) were calculated using the CORR procedure of SAS. Differences between least square means were determined with the PDIFF option of SAS. Significances were declared at $P < 0.05$ and tendencies considered when $P < 0.10$.

3.4 RESULTS AND DISCUSION

3.4.1 Fleece Extension and Wool Weight

At d 100 of pregnancy, after approximately 15 mo of the previous yearly shearing, fleece covered 13% more body surface in MN than LC ewes, as indicated by their extension scores (2.11 ± 0.06 vs. 1.87 ± 0.06 points, respectively; $P < 0.001$). After shearing, clipped wool weight in the S100 ewes was 45% greater in MN than LC (2.43 ± 0.20 vs. 1.68 ± 0.32 kg/ewe, respectively; $P = 0.048$). Wool production was equivalent to 3.33 ± 0.04 and 2.20 ± 0.05 kg wool/100 kg BW for MN and LC, respectively ($P < 0.001$) in the range of values reported by Smoliak and Slen (1972) in Rambouillet and Corriedale ewes under different grazing conditions. Correlations between wool weight and fleece extension score were positive for both breeds which, on average, produced 1.15 (r = 0.69; $P < 0.05$) and 0.90 (r = 0.67; $P < 0.05$) kg wool per point of fleece score, for MN and LC, respectively. These results agreed with those of Elhadi et al. (2019) for whom MN ewes shorn in the middle of lactation had greater wool production and fleece extension than LC ewes.

Given the similar body frame and close BW of both breeds, greater alleviation of heat

production was expected in the MN ewes, after performing the pregnancy shearing, in comparison to LC ewes. Elhadi et al. (2019) also reported a greater decrease of rectal temperature in MN ewes, when compared to shorn LC ewes, when shorn under milk-winter conditions.

3.4.2 Respiratory Rate

Changes of RR by shearing treatment at different ambient temperatures are shown in Figure 3.2. Ambient temperatures had dramatic effects on RR of sheep (Silanikove, 2000) which varied exponentially with temperature in our data ($r^2 = 0.90$; $P < 0.001$) passing from 42 ± 2 bpm to 94 ± 6 bpm (+124%; $P < 0.001$), when temperature increased from 20 to 28°C. Even though the fleece covered more body surface in MN ewes, as above indicated, no differences in RR were found by breed ($P = 0.68$). Shearing treatment affected the RR ($P = 0.037$) and an interaction was observed between shearing × temperature ($P < 0.001$). Thus, no differences on RR by shearing treatment were detected at 20 and 25ºC, whereas RR values were 37% lower in S100 than in SBB and CO ewes at 28ºC (68 ± 7 vs. 108 ± 8 bpm, on average; $P < 0.001$), the last no differing between them ($P = 0.11$).

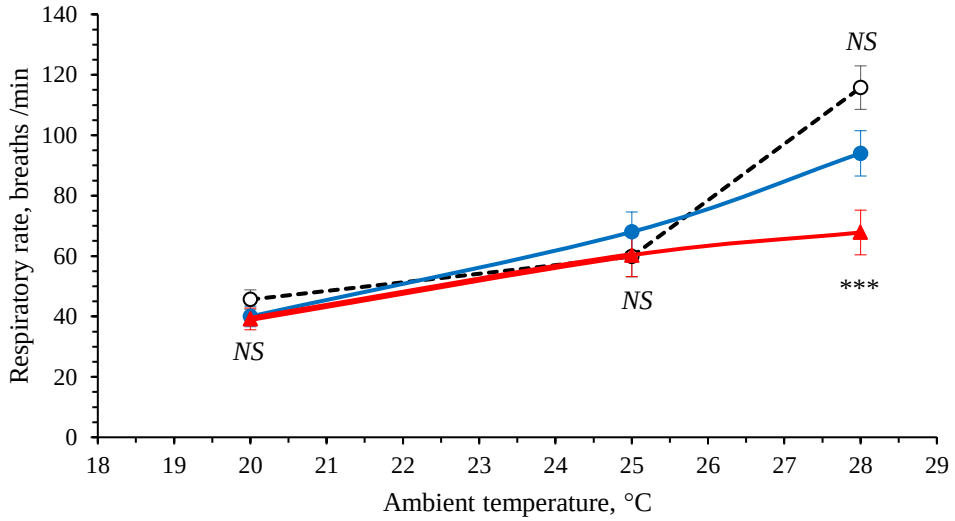


Figure 3.2. Respiratory rate of pregnant Manchega and Lacaune dairy ewes (breeds confounded) by shearing treatment: according to shearing treatment: ○ (CO, control, open circle, and dashed line), ● (SBB, shorn before mating, closed circle, and solid blue line), and ▲ (S100, shorn at d 100 of pregnancy, closed triangle, and solid red line) at different ambient temperatures: 20, 25 and 28 °C. Values are means with the SEM indicated by vertical bars.

3.4.3 Blood Indicators

Plasmatic values of the ewes at lambing are shown in Table 3.1. Shearing strategy only affected the glycemia of the MN ewes (P = 0.008), in which the SBB and S100 shearing treatments increased, on average, 86% the plasma values of glucose when compared to those of the CO ewes ($P < 0.05$). Similarly, in the LC ewes, SBB and S100 treatments increased 46% the glycemia values, on average and compared to CO ewes, although the effect was not significant (P = 0.13). Normal values of glycemia in sheep usually range between 50 and 80 mg/dL (Fielder, 2021), with few differences during pregnancy by effect of litter size. Moallen et al. (2012) reported values ranging between 45 and 52 mg/dL in Assaf ewes carrying 4 to 1 lamb, respectively, at late pregnancy.

Table 3.1. Effects of shearing treatments on the metabolic indicators in plasma at parturition of 2 breeds of dairy ewes (data are LSM).

	Shearing treatments[1]				Effect (P-value)	
Breed and item	CO	SBB	S100	Mean ± SE	ST[1]	LS[2]
Manchega						
Glucose, mg/dL	59[b]	113[a]	107[a]	93 ± 14	0.008	0.73
Insulin, ng/mL	0.629	1.021	0.703	0.784 ± 0.137	0.11	0.001
NEFA[3], mmol/L	0.717	0.927	0.740	0.795 ± 0.116	0.37	0.10
BHB[4], mmol/L	0.597	0.610	0.612	0.606 ± 0.084	0.98	0.007
Lacaune						
Glucose, mg/dL	69	89	113	90 ± 15	0.13	0.30
Insulin, ng/mL	0.974	1.070	1.036	1.027 ± 0.271	0.96	0.032
NEFA[3], mmol/L	0.613	0.568	0.607	0.596 ± 0.086	0.91	0.041
BHB[4], mmol/L	0.524	0.507	0.450	0.494 ± 0.072	0.72	0.23

[a, b] Different letters in the same line indicate differences between treatments at $P < 0.05$.
[1]Shearing treatment (CO, control; SBB, shorn before breeding; S100, shorn at d 100 of pregnancy); [2]litter size; [3]non-esterified fatty acids; [4]β-hydroxybutyrate.

On the other hand, glycemia dramatically change around lambing with the aim of coping with the high glucose uptake in the mammary gland for the synthesis of lactose. Thus, Banchero et al. (2004) reported peaks of glycemia between 100 and 160 mg/dL in recently lambed Corriedale ewes during the first 10-h after lambing. These high post-lambing glycemia values were also observed in our shorn ewes (i.e., SBB and S100), but not in the CO ewes which remained at normal range (Figure 3.3). No effects in glycemia were detected due to lambing size nor its interaction with shearing in both breeds (Table 3.1). Agreeing this, SBB and S100 ewes showed numerically greater concentrations of lactose in colostrum than CO ewes, on

average, although the effects of shearing were not significant (MN, 8%, $P = 0.68$; LC, 7%, $P = 0.18$; data not shown).

No other authors, in our knowledge, have reported glycemia values of ewes shorn at late pregnancy immediately after parturition. Nevertheless, Symonds et al. (1986), as previously indicated, observed numerically increased plasmatic glucose after shearing, which is supported by the significant results of Rosales-Nieto et al. (2020) in Polypay × Dorset ewes shorn at d 100 and monitored from shearing up through lambing.

On the other hand, in parallel to the increasing effects of shearing on glycemia after lambing, plasmatic insulin values were numerically greater in the shorn ewes when compared to CO (Figure 3.3). No differences were detected between treatments in both breeds (MN, $P = 0.11$; LC, $P = 0.96$; Table 3.1). Although there are no data on plasmatic insulin after parturition of shorn-pregnant ewes, Symonds et al. (1986) and Revell et al. (2000) reported a tendency or a significant decrease of insulin before lambing, respectively, which are contrary to our results.

Despite no differences were detected on the glycemia of the ewes by effect of litter size in either breed (Table 3.1), twin-bearing MN ewes had less insulin (−157%) than single-bearing ewes (0.439 ± 0.108 vs. 1.130 ± 0.111 ng/mL, respectively, $P < 0.001$). The effect was consistent and similar (−98%) in twin-bearing LC ewes compared to single-bearing ewes (0.690 ± 0.160 vs. 1.363 ± 0.241 ng/mL, respectively, $P < 0.001$).

These results agree with those Moallen et al. (2012) who observed decreases of plasmatic insulin at late pregnancy, the larger the litter size (from 1 to 4 lambs) the lower the insulinemia (41 and 80% those of single bearing ewes). The positive effects of insulin, as growth factor and lipogenic hormone (Etherton and Evock, 1986; Qaid and Abdelrahman, 2016), may be one of the reasons for recommending the use of shearing at late pregnancy in sheep. We hypothesised that an increase in plasmatic insulin in the ewes may increase the content of insulin in colostrum, which should be beneficial specially for twin and low-birthweight lambs. The decrease of plasma insulin observed in our twin-bearing ewes agrees with the 94% insulin decrease by Rumball et al. (2008) in the plasma of twin-bearing ewes submitted to glucose challenges in late pregnancy.

Moreover, the insulin decrease by twin-bearing was larger in SBB (−302%, 0.407 ± 0.153 vs. 1.635 ± 0.229 ng/mL, $P < 0.001$) and CO (−267%, 0.269 ± 0.173 vs. 0.988 ± 0.187 ng/mL, $P = 0.008$) ewes, than in S100 where it was not detectable (0.640 ± 0.229 vs 0.767 ± 0.153

ng/mL, *P* = 0.65) ewes, as shown in Figure 3.4. A significant interaction between shearing treatment × litter size was detected for plasma insulin, which may be consequence of the few numbers of twin-bearing ewes of the S100 treatment.

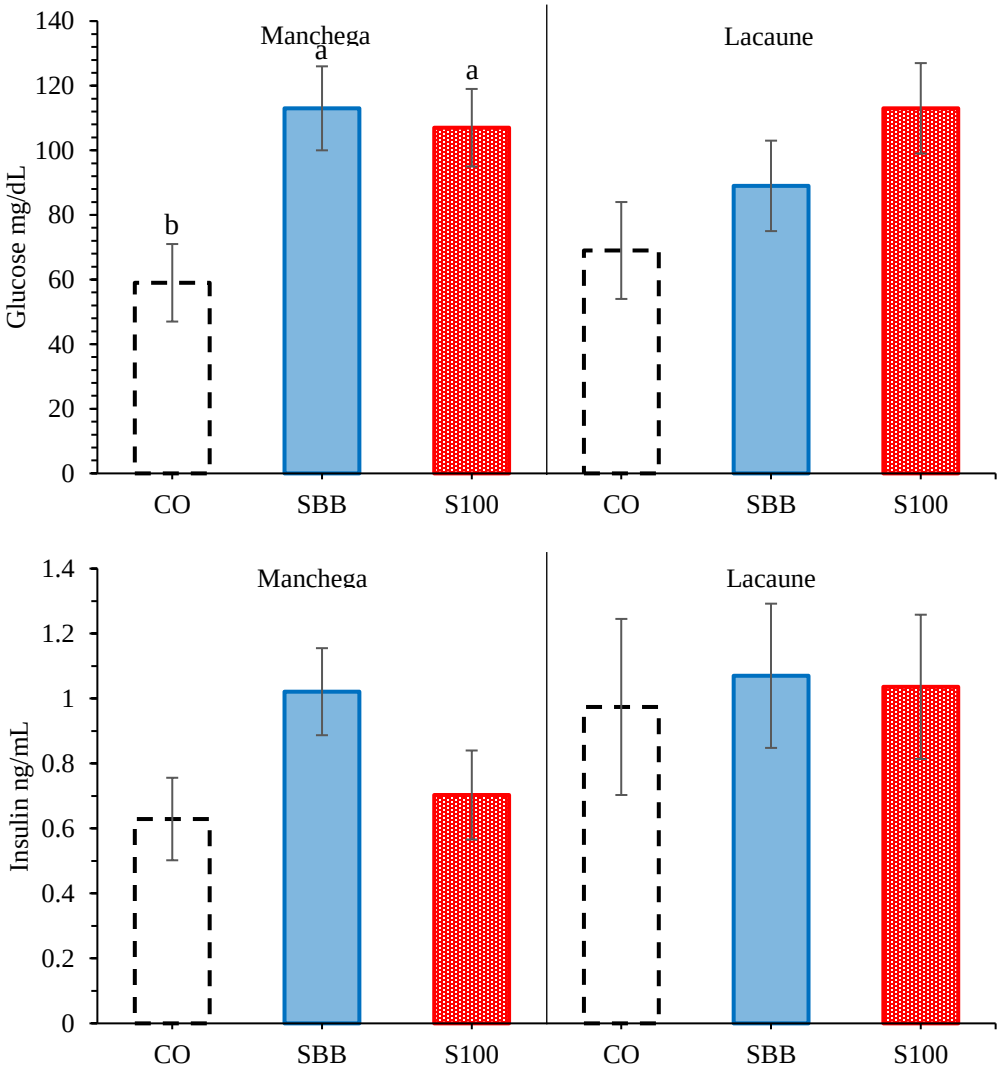


Figure 3.3. Plasmatic glucose and insulin values of Manchega (MN) and Lacaune (LC) dairy ewes at lambing according to shearing treatment (CO, control, dashed line; SBB, shorn before mating, and S100, shorn at d 100 of pregnancy). Values are means with the SEM indicated by vertical bars.

No differences in the plasmatic values of NEFA (*P* = 0.37 to 0.91) or BHB (*P* = 0.72 to 0.98) were detected among treatments in both breeds (Table 3.1), indicating a similar adipose tissue mobilization and energy balance at lambing of the ewes submitted to the different shearing

strategies. Nevertheless, BHB values were greater in twin- (54%) than single-bearing MN ewes (0.735 ± 0.059 vs. 0.478 ± 0.067 mmol/L, respectively, $P = 0.007$), whereas NEFA were 43% greater in twin- than single-bearing in the LC ewes (0.702 ± 0.057 vs. 0.490 ± 0.079 mmol/L, $P = 0.041$). These results agree with those reported by Rumball et al. (2008) in Romney ewes at late pregnancy.

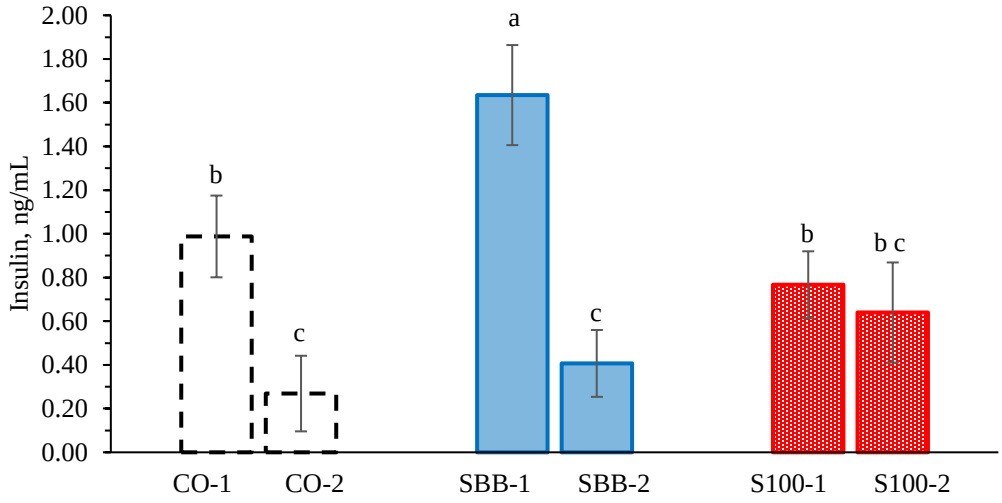


Figure 3.4. Plasmatic insulin values of Manchega dairy ewes at lambing according to shearing treatment (CO, control, dashed line; SBB, shorn before mating, and S100, shorn at d 100 of pregnancy) and litter size (1, single bearing ewes and 2, twin bearing ewes). Values are means with the SEM indicated by vertical bars.

The NEFA mean values observed in our ewes after parturition exceeded the reference range for sheep (0.10 to 0.50 mmol/L; Reintke et al., 2021) which is consistent with the increased energy requirements of the late pregnancy as reported previously. Nevertheless, the maternal BHB levels found in our ewes at lambing (< 0.8 mmol/L) reflected a tolerable negative balance according to Sargison (2007) and Crilly et al. (2021). Considering the glucose results above indicated for MN ewes, it seems that our shorn MN ewes (SBB and S100) prioritized their foetus and uterine tissues to be better adapted at lambing than the CO did. The metabolic adjustments done were different depending on litter size, particularly in the case of insulin in both breeds, BHB in the MN ewes, and NEFA in the LC ewes.

3.4.4 Lamb Weight and Growth

On the contrary to data previously reported in meat or wool ewes shorn pregnant under winter-housing conditions (Rutter et al., 1971, 1972; Revell et al., 2000) or grazing during the day and bearing singletons (Cam and Kuran, 2004), no effects of shearing treatments were detected on lamb birthweight in our MN (P = 0.44) and LC (P = 0.37) dairy ewes (Table 3.2). Moreover, no effects of the interaction treatment × litter size were detected in our data. Cam and Kuran (2004) did not report differences in twin-lamb's birthweight, whereas the lack of effect was reported by García-Rodríguez et al. (2012) in singletons from Latxa dairy ewes. On the contrary, Cam and Kuran (2004) reported differences in singletons (19%) and García-Rodríguez et al. (2012) in twins (14%) birthweight, respectively, both under winter conditions.

These results were not observed under summer conditions, like in our data. Therefore, Leivobich et al. (2011) only detected differences on lamb birthweight of Assaf dairy ewes shorn at late pregnancy (d −30) when shearing was associated to cooling, but they did not consider the effect of litter size.

Table 3.2. Effects of shearing treatments on lamb growth during suckling in 2 breeds of dairy ewes (data are LSM).

	Shearing treatment[1]			Mean ± SE	Effect (P-value)	
Breed and item	CO	SBB	S100		ST[1]	LS[2]
Manchega						
Birthweight, kg	4.41	4.39	4.25	4.35 ± 0.56	0.44	0.006
Weaning weight[3], kg	11.92	10.96	11.58	11.47 ± 0.56	–	–
Suckling gain, kg	7.44	6.57	7.33	7.10 ± 0.46	0.45	0.001
Average daily gain, g/d	248	219	244	237 ± 15	0.45	0.001
Lacaune						
Birthweight, kg	4.00	3.89	3.96	3.95 ± 0.48	0.37	0.003
Weaning weight[3], kg	11.58	11.16	12.39	11.69 ± 0.48	–	–
Suckling gain, kg	7.58	7.29	8.34	7.72 ± 0.61	0.69	0.001
Average daily gain, g/d	253	243	278	257 ± 21	0.69	0.001

[1]Shearing treatment (CO, control; SBB, shorn before breeding; S100, shorn at d 100 of pregnancy); [2]litter size; [3]weaned at constant age (d 28).

The birthweight response to pregnancy shearing seems to be most consistent when the ewes are shorn early (in mid-pregnancy) and carrying twins, as reviewed by Kenyon et al. (2003). In this regard, Morris and McCutcheon (1997) reported heavier twin lambs born from Border Leicester × Romney ewes shorn at d 70, 100 and 130 of pregnancy vs. unshorn, but the greatest

birthweight increase (0.7 kg/lamb) was achieved when the ewes were shorn at d 70 of pregnancy. Similarly, Banchero et al. (2010) found that twin-bearing Corriedale ewes shorn at d 70 or d 120 of pregnancy, showed greater increases on lamb birthweight (0.8 kg and 0.3 kg/lamb, respectively), heavier placentas and lower number of cotyledons when shorn earlier, compared to unshorn twin-bearing control ewes. This may be a consequence of the increase of glucose and insulin concentrations produced by pregnancy shearing, as observed numerically in our MN ewes bearing twins of treatments CO and S100 for glucose (56 ± 16 vs. 123 ± 19 mg/dL, respectively, $P = 0.011$) and insulin (0.269 ± 0.173 vs. 0.640 ± 0.229 ng/mL, respectively, $P = 0.21$; Figure 3.4). No effects of shearing treatments on growth performances during suckling as well as on weaning weight were detected in the lambs of our ewes of both breeds ($P = 0.45$ to 0.69; Table 3.2). On the contrary, Cam and Kuran (2004) reported greater lamb growth and weaning weigh (20%) during suckling in the shorn pregnant ewes. No data of lamb growth were reported by Rutter et al. (1971, 1972), Revell et al. (2000), Banchero et al. (2010) and García-Rodríguez et al. (2012). Although, there is not a general agreement on the benefits of pregnancy shearing in all seasons and litter size conditions, no negative data has been reported except in the case of poor feeding conditions, cold and rainy weather, and twin-bearing ewes (Ehrhardt, 2021).

3.4.5 Colostrum

Shearing treatments did not affect colostrum components in either breed ($P = 0.11$ to 0.71). Although no direct comparison was done in our data, MN colostrum (fat, 8.28 ± 0.71%; protein, 20.32 ± 1.32%, true protein, 20.05 ± 1.40%; casein, 6.90 ± 0.35%; lactose, 2.36 ± 0.22%; total solids, 31.9 ± 1.5%; on average) showed greater component content than LC (fat, 4.55 ± 0.71%; protein, 13.45 ± 1.22%; true protein, 12.91 ± 1.24%; casein, 5.04 ± 0.49%; lactose, 2.79 ± 0.24%; total solids, 22.4 ± 1.5%; on average). True protein and casein contents accounted for 97% and 36% of total protein, respectively, on average and in both breeds. As previously indicated, lactose content in colostrum was low in both breeds, but its content was numerically greater in the shorn than the CO ewes. Our results agree with those of Banchero et al. (2010) who did not find effects of shearing treatments in colostrum yield and composition of Corriedale ewes, although colostrum protein content tended to increase in the earliest shorn ewes (d 70). Moreover, according to Banchero et al. (2010), lactose content was greater in the twin-bearing

ewes shorn at d 120 in comparison to unshorn ewes (1.9 vs. 1.5%, respectively); no effects were reported for singleton-bearing ewes (Banchero et al., 2010). This effect of litter size was not detected in our data. Correspondingly, no differences on colostrum density were also observed in our data between shearing treatments in MN (1.075 ± 0.005 g/mL, on average, $P = 0.37$) and LC (1.060 ± 0.005 g/mL, on average, $P = 0.63$) ewes, but positive and significant correlations ($P < 0.05$) were obtained in both breeds for colostrum density and total solids (MN, r = 0.51; LC, r = 0.45), true protein (MN, r = 0.70; LC, r = 0.58) and casein (MN, r = 0.33; LC, r = 0.50) contents, but negative for lactose content in MN (r = –0.45) and no significant in LC ewes.

More importantly, IgG contents were similar between shearing treatments in both breeds (MN, 30.6 ± 4.7 mg/mL, on average; $P = 0.50$) and LC (20.8 ± 3.7 mg/mL; $P = 0.29$), indicating no differences in the passive immunity transferred to lambs. Both mean values of IgG were high according to Kessler et al. (2019) and correlated positively ($P < 0.05$) with colostrum protein in both breeds (MN, r = 0.58; LC, r = 0.58). Regarding the effect of litter size, twin-bearing MN ewes showed lower IgG content than single-bearing ewes (24.03 ± 3.22 vs. 37.19 ± 3.77 mg/mL, respectively, $P = 0.023$). No differences were detected in the case of LC ewes ($P = 0.76$).

Insulin content in colostrum was also high, compared to plasma values (ng/mL = µg/L), and did not differ between treatments in the MN (16.96 ± 2.81 µg/L, on average, $P = 0.52$) and the LC (13.66 ± 4.93 µg/L, on average, $P = 0.40$) ewes. Interestingly, insulin values in colostrum exceeded more than 20-fold in MN and 10-fold in LC the values found in plasma (Table 3.1), implicating an active transport through the blood-mammary barrier (Einspanier and Schams, 1991; Nowak et al., 1994). According to Nowak et al. (1994), the high values of insulin detected in colostrum may be an important mechanism for the transient deficit of this hormone during the first hours of life of newborn mammals. Litter size also affected colostrum insulin content, like previously reported in plasma, and twin-bearing MN ewes showed lower insulin content than single-bearing ewes (13.22 ± 1.88 vs. 20.69 ± 2.50 µg/L, respectively, $P = 0.042$). No differences were detected in the case of LC ewes ($P = 0.92$).

3.4.6 Milk Yield during Suckling

The effects of shearing treatments on milk yield of MN and LC ewes for the entire lactation is shown in Figure 3.5, in which the suckling and milking periods are distinguished. Despite the

large differences observed between MN and LC ewes, as previously reported during the suckling period (Flores et al., 2008), the estimated amount of milk produced by the ewes until the weaning of the lambs did not vary by effect of shearing treatments in MN (2.47 ± 0.14 kg/d, P = 0.60, Figure 3.5a) and LC ewes (2.76 ± 0.26 kg/d, P = 0.83, Figure 3.5b). The lack of shearing effect may be partially explained by the absence of differences in lamb birthweight between treatments, which may suggest that mammary gland was stimulated similarly. Cam and Kuran (2004) reported 30% increase in milk yield during suckling of Karayaka ewes shorn pregnant (d 100) when compared to unshorn ewes. Similar results were found by Sphor et al. (2011) in Polwarth ewes, who reported 22% more milk yield during suckling in shorn ewes at mid-pregnancy (d 53) when compared to unshorn. The authors attributed these results to the lambs either directly (weight) or indirectly (behavior). However, Banchero et al. (2010), claimed that vigor of Corriedale lambs (first hour of life) increased when the ewes were shorn in mid (d 70) and late pregnancy (d 120), independently of the birthweight and that it could be related to the possible greater physiological development of lambs.

3.4.7 Milk Composition during Suckling

Large differences on milk composition of MN and LC ewes during suckling were also reported by Flores et al. (2008), inversely related to their respective milk yield, and the effect of shearing strategies on milk composition during suckling was studied separately for each breed. Regarding MN ewes (Table 3.3), the S100 treatment increased milk protein (6%, P = 0.049), casein (6%, P = 0.037) and tended to increase true protein (6%, P = 0.07) in milk contents when compared to CO ewes, without differences with SBB ewes (P = 0.32 to 0.44). No differences were detected on milk contents of total solids, fat and lactose by shearing treatment (P = 0.28 to 0.93). On the other hand, the S100 treatment increased total milk solids in the LC ewes (8%, P = 0.040), compared to SBB treatment (Table 3.3), and milk fat content (8%, P = 0.031) when compared to SBB. No differences between treatments were detected for total protein, true protein, casein and lactose contents (P = 0.36 to 0.46) in the LC ewes. In contrast to our results, Sphor et al. (2011) in grazing Polwarth ewes shorn at d 53 of pregnancy and producing 22% more milk as above indicated, reported no differences in milk fat composition and lower milk protein content (samples obtained with the same methodology than in our study), when

compared to unshorn ewes. Despite the lack of differences on milk composition between treatments, the birth and weaning weights of the lambs were greater in the ewes shorn pregnant (Sphor et al., 2011). In our ewes of both breeds, the differences detected in milk composition during the suckling period, were not enough to trigger changes in lamb growth during suckling or in weaning weight, as mentioned above.

Table 3.3. Effects of shearing treatments on milk composition during suckling and milking periods in 2 breeds of dairy ewes (data are LSM).

	Shearing treatment[1]			Mean ± SE	Effect (*P*-value)	
Breed and item	CO	SBB	S100		ST[1]	LS[2]
Manchega						
Suckling (d 5 to 28)						
Total solids, %	18.70	18.52	18.58	18.54 ± 0.38	0.93	0.34
Fat, %	8.39	8.15	8.02	8.16 ± 0.37	0.73	0.46
Total protein, %	4.99^{b}	5.19^{ab}	5.29^{a}	5.10 ± 0.09	0.049	0.06
True protein, %	4.50^{y}	4.68^{xy}	4.77^{x}	4.60 ± 0.09	0.07	0.08
Casein, %	3.72^{b}	3.86^{ab}	3.95^{a}	3.81 ± 0.07	0.037	0.11
Lactose, %	4.33	4.17	4.26	4.25 ± 0.07	0.28	0.18
Milking (d 35 to 77)						
Total solids, %	17.73	18.14	18.21	18.01 ± 0.25	0.25	0.092
Fat, %	6.75	7.06	7.14	6.98 ± 0.21	0.30	0.41
Total protein, %	5.68	5.73	5.94	5.77 ± 0.15	0.15	0.001
True protein, %	5.35	5.41	5.60	5.44 ± 0.11	0.18	0.001
Casein, %	4.42	4.45	4.60	4.48 ± 0.09	0.19	0.002
Lactose, %	4.33	4.25	4.20	4.26 ± 0.06	0.34	0.036
Lacaune						
Suckling (d 5 to 28)						
Total solids, %	17.68^{ab}	16.96^{b}	18.30^{a}	17.68 ± 0.55	0.040	0.11
Fat, %	7.59^{ab}	6.65^{b}	7.85^{a}	7.44 ± 0.37	0.031	0.032
Total protein, %	5.11	5.15	5.35	5.14 ± 0.14	0.36	0.91
True protein, %	4.64	4.71	4.87	4.68 ± 0.14	0.45	0.89
Casein, %	3.81	3.82	3.99	3.82 ± 0.11	0.38	0.60
Lactose, %	4.00	4.17	4.11	4.09 ± 0.10	0.46	0.21
Milking (d 35 to 77)						
Total solids, %	16.82	16.41	17.08	16.79 ± 0.42	0.42	0.70
Fat, %	5.80	6.34	6.25	6.18 ± 0.33	0.40	0.35
Total protein, %	5.29	5.22	5.60	5.34 ± 0.25	0.23	0.80
True protein, %	4.98	4.89	5.27	5.02 ± 0.25	0.23	0.82
Casein, %	4.08	4.03	4.28	4.11 ± 0.19	0.31	0.81
Lactose, %	4.19	4.38	4.23	4.27 ± 0.10	0.30	0.34

[a,b]Different letters in the same line indicate differences between treatments at $P < 0.05$; [x, y]Different letters in the same line indicate tendency to differ between treatments at $P < 0.10$; [1]Shearing treatment (CO, control; SBB, shorn before breeding; S100, shorn at d 100 of pregnancy); [2]Litter size.

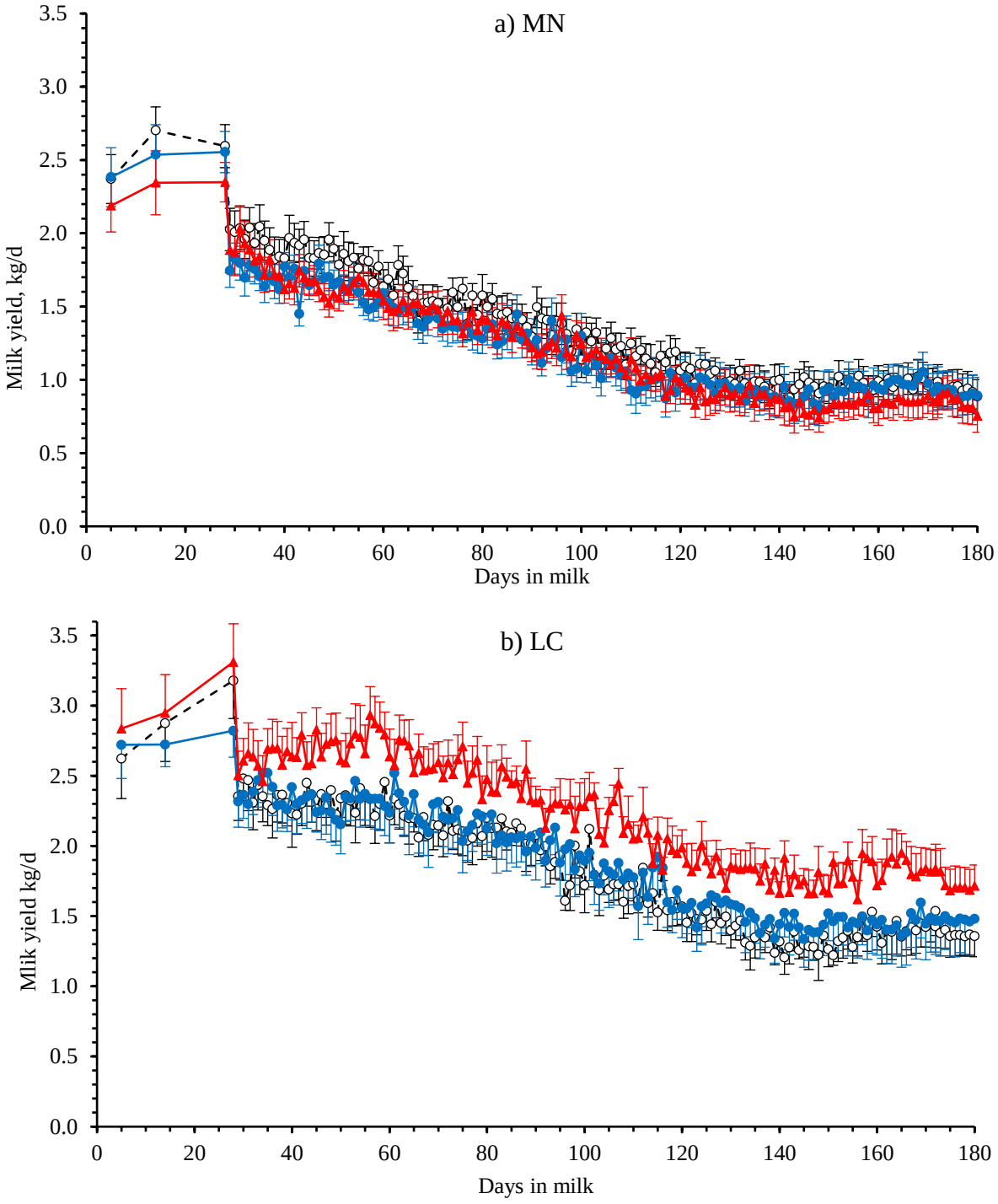


Figure 3.5. Milk yield of Manchega (MN) and Lacaune (LC) dairy ewes by shearing treatment: ○ (CO, control, open circle and dashed line), ● (SBB, shorn before mating, closed circle and solid blue line), and ▲ (S100, shorn at d 100 of pregnancy, closed triangle and solid red line). Values are means with the SEM indicated by vertical bars.

3.4.8 Milk Yield during Machine Milking

The effects of shearing treatments on milk yield during the milking period (after the weaning of the lambs) varied according to ewe breed, as shown in Figure 3.5. First, similar lactation curves and average milk yield among shearing treatments was observed in the MN ewes (1.23 ± 0.09 kg/d, on average, $P = 0.52$). Our results agree with those of García-Rodríguez et al. (2012) in Latxa ewes shorn at late-pregnancy (d 110). Milk yield of our MN ewes showed a linear descending pattern from weaning (d 28) to approximately d 150 ($y = –0.0091 x + 2.130$, $r^2 = 0.98$, $P < 0.001$), the linear persistency coefficient being –9.1 g/d. Thereafter, lactation curve reached a plateau until d 180 (0.90 ± 0.07 kg/d), which was a consequence of the positive effect of the spring photoperiod at the end of lactation, as usually observed under our management conditions (Figure 3.5a). Second, in the case of LC ewes (Figure 3.5b), the S100 treated ewes tended to yield 28% more milk than CO (2.19 ± 0.17 vs. 1.71 ± 0.17 kg/d, respectively, $P = 0.08$) which was consistent with the positive effect (10%) reported by Elhadi et al. (2019) in LC ewes shorn during milking and winter conditions. Nevertheless, no differences were detected between SBB (1.99 ± 0.17 kg/d) and the CO and S100 treatments in the LC ewes. Milk yield of our LC ewes also showed a linear descending pattern from 28 to d 150 ($y = –0.0098 x + 2.936$, $r^2 = 0.94$, $P < 0.001$), the linear regression coefficient being –9.8 g/d and similar to that of the MN ewes previously indicated. The impact of the spring photoperiod at the end of lactation was also observed in the case of LC ewes, with a plateau of 1.55 ± 0.11 kg/d after d 150. The milk yield response to shearing treatments in our LC was greater than reported by Leibovich et al. (2011, 7.4%) in Assaf ewes shorn in late pregnancy (d 118) under summer conditions and with barn cooling.

Positive correlations were detected between milk yield values from suckling and milking periods (MN, $r = 0.35$, $P = 0.020$; LC, $r = 0.46$, $P = 0.011$), indicating that the ewes performed similarly in both periods. The correlations improved (MN, $r = 0.70$, $P < 0.001$; LC, $r = 0.55$, $P = 0.002$) when the milk yield at the 1st milk recording (d 5) was correlated with those of the rest of suckling (d 14 to 28), but the correlations were no significant ($P = 0.09$ to 0.16) when correlated with milk yield during milking, supporting that the effect of treatments was consistent throughout the study.

3.4.9 Milk Composition during Machine Milking

Composition of the machine milked milk (Table 3.3) did not change by effect of shearing treatments either in MN (*P* = 0.15 to 0.34) and LC ewes (*P* = 0.23 to 0.42). These results were in accordance with García-Rodríguez et al. (2012) who found no significant changes in the fat and protein contents of machine-milked milk of Latxa ewes shorn (d 110 of pregnancy, on average) or unshorn under winter and housing conditions. On the other hand, our results disagree with Knight et al. (1993), who detected an increase in fat, protein and total solids of the milk of Dorset ewes shorn in late pregnancy (d −12 to −52) under winter conditions. Moreover, Leibovich et al. (2011) reported 11% more fat and 3% more protein milk contents in Assaf ewes machine-milked from lambing that were shorn pregnant (d –30 of pregnancy) with barn cooling in comparison to unshorn ewes.

The similar milk composition between shearing treatments in our ewes during machine milking indicated no detrimental effects of pregnancy shearing on milk synthesis, which was related with the no significant variations in milk yield observed in our results, as previously mentioned.

3.4.10 Body Reserves

Despite the differences in fleece weight (MN, 2.43 ± 0.20 kg) and gravid uterus accretion according to prolificacy (MN, 1.57 ± 0.21 lambs/ewe) during late-pregnancy (d −43 to −4), BW of MN ewes increased (3.73 ± 0.39 kg BW, $P < 0.001$) but did not vary by shearing treatment (74.03 ± 1.36 kg, on average, *P* = 0.09 to 0.86). Nevertheless, differences by shearing treatment were observed on body fatness of the S100 ewes, that had greater BCS (3.16 ± 0.11 units, *P* = 0.001 to 0.004, respectively) than the CO and SBB ewes (2.71 ± 0.11 and 2.75 ± 0.12 units, respectively), during the same late-pregnancy period (Figure 3.6a). This positive effect of shearing at d 100 agreed with the greater glycemia at lambing previously indicated in Table 3.1 and with the improved metabolic adaptations to fat mobilization without NEFA increase, as reported by Symonds et al. (1989). No differences in BCS between shearing treatments were detected at lambing (MN, 2.60 ± 0.07 units, on average) which fits within the 2.5 to 3.0 range recommended by Kenyon et al. (2014) for sheep.

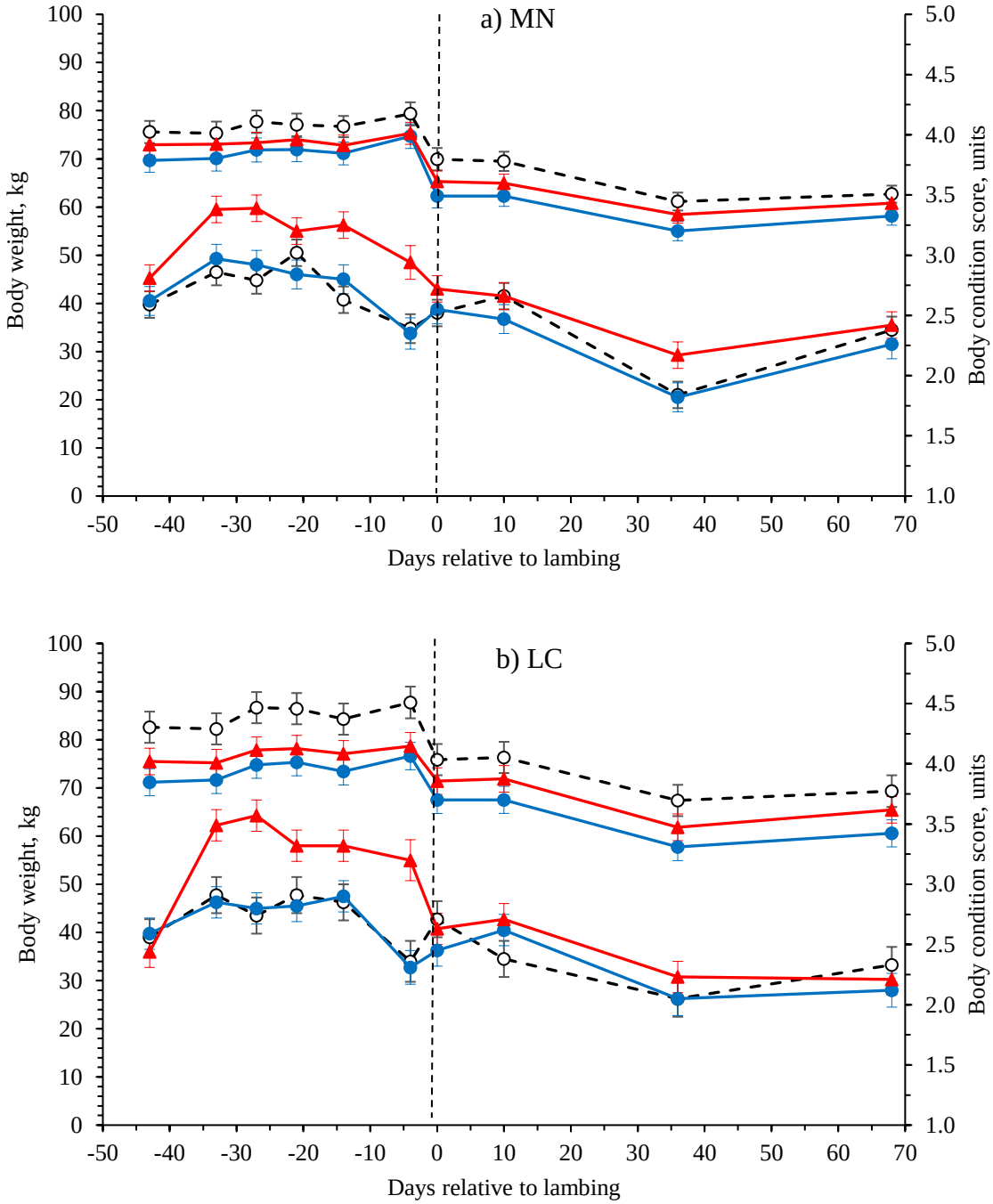


Figure 3.6. Body weight and body condition score of Manchega (MN) and Lacaune (LC) dairy ewes by shearing treatment: ○ (CO, control, open circle and dashed line), ● (SBB, shorn before mating, closed circle and solid blue line), and ▲ (S100, shorn at d 100 of pregnancy, closed triangle and solid red line). Values are means with the SEM indicated by vertical bars.

Compared to d –4, all MN ewes lost BW at lambing (10.61 ± 0.53 kg, on average, $P < 0.001$), although the CO were heavier than SBB at lambing (69.94 ± 2.32 vs. 62.29 ± 2.48 kg, $P = 0.030$) and did not differ from S100 ewes (65.31 ± 2.19 kg, $P = 0.16$). The BW of CO ewes continued being heavier than SBB ($P = 0.020$ to 0.032) until d 36 postlambing but did not differ from S100 throughout the whole postlambing period (Figure 3.5a, $P = 0.20$ to 0.45). The BCS of MN ewes continued to decrease after lambing without differences between shearing treatments (−0.24 ± 0.06 units, on average), except at d 36, where S100 was greater (2.17 ± 0.11 units, $P = 0.041$ and 0.036, respectively) than CO and SBB treatments (1.83 ± 0.12 units, on average, $P = 0.91$), as shown in Figure 3.5a.

Similar effects of shearing treatments on body reserves were observed in the LC ewes, although in this case the differences between S100 and CO were greater during late-pregnancy period (Figure 3.5b). Thus, despite the lower fleece weight (LC, 1.68 ± 0.32 kg) and slightly greater gravid uterus accretion according to prolificacy (LC, 1.67 ± 0.27 lambs/ewe) during late-pregnancy (d −43 to −4), the BW of LC ewes increased (4.58 ± 0.88 kg BW, $P < 0.001$) with differences due to shearing treatment. The BW of CO ewes was greater than SBB during late-pregnancy ($P = 0.006$ to 0.014), at lambing ($P = 0.052$) and postlambing ($P = 0.027$ to 0.044) periods, but only tended to differ from S100 ewes ($P = 0.06$ to 0.10).

The differences in BW observed at lambing were maintained throughout the whole postlambing period with a negative trend from lambing (71.60 ± 1.70 kg BW, on average) to d 68 (65.13 ± 1.71 kg BW, on average) for all ewe groups (Figure 3.5b). Moreover, differences by shearing treatment were observed on body fatness of the S100 ewes, that had greater BCS (3.22 ± 0.13 units, $P = 0.008$ and 0.001, respectively) than the CO and SBB ewes (2.71 ± 0.17 and 2.72 ± 0.14 units, respectively), during the same late-pregnancy period (Figure 3.5b). No effect of shearing at d 100 was observed in the glycemia at lambing of LC ewes (Table 3.1). Compared to d−4, likely it was shown in MN ewes, all LC ewes lost BW at lambing (9.41 ± 0.65 kg, on average, $P < 0.001$), although the CO were heavier than SBB at lambing (75.88 ± 3.24 vs. 67.50 ± 2.81 kg, $P = 0.052$, respectively) and did not differ from S100 (71.41 ± 2.76 kg, $P = 0.29$) in the LC ewes.

The BW of CO ewes continued being heavier than SBB ($P = 0.027$ to 0.044) until d 68 postlambing but did not differ from S100 throughout the whole period (Figure 3.5b, $P = 0.19$ to 0.37). The BCS of LC ewes also decreased after lambing, without differences between shearing

treatments (−0.35 ± 0.09 units, on average) as shown in Figure 3.5b. Values of BCS at lambing were also acceptable for the LC ewes (LC, 2.60 ± 0.08 units, on average) according to Kenyon et al. (2014), although a greater value should be recommendable given their greater milk performance, when compared to the MN ewes.

The obtained results showed that S100 ewes had greater fatness than CO and SBB ewes at late-pregnancy, in both MN and LC ewes, but once lambing occurred the differences disappeared as also reflected by their similar NEFA and BHB plasmatic values as early mentioned (Table 3.1). Our results support the hypothesis that shorn ewes are better adapted to utilize body fat reserves as energy source and to maintain glycemia during late-pregnancy, reducing the levels of plasmatic NEFA and the risks of clinical ketosis, as indicated by Symonds et al. (1989) under winter conditions.

3.4.11 Milk Coagulation Traits

Milk composition and coagulation traits of the milk in late lactation (d 160) are shown by ewe's breed in Table 3.4. To our knowledge, no study has assessed the effect of shearing ewes on milk coagulation traits.

Agreeing the lack of differences in milk composition during the machine milk period (d 35 to 77; Table 3.3), no compositional differences of milk batches used for assessing the milk coagulation traits were detected by shearing treatment (Table 3.4; $P = 0.47$ to 0.96; LC, $P = 0.13$ to 0.46). Consequently, no differences in curd firmness, expressed as F45 (mm), were observed in the milk of either MN (46.0 ± 1.5 mm, $P = 0.53$) or LC (42.6 ± 2.8 mm, $P = 0.41$) ewes by effect of shearing treatments. On the contrary, milk coagulation time, expressed as RCT (min), varied according to shearing treatments but, interestingly, with effects of opposite sign depending on ewe's breed (Table 3.4). Thus, in the case of the MN ewes, the S100 treatment delayed the formation of curd (–9%; $P = 0.003$), whereas in LC it was enhanced (8%; $P = 0.009$), when compared to their respective CO ewes. On the other hand, no effects of SBB were detected in either breed with regard to CO ewes (MN, $P = 0.21$; LC, $P = 0.52$).

Nevertheless, other compositional and physic-chemical factors of milk such as Ca concentration, SCC, pH, or casein micelle characteristics could be associated to the RCT results according to Pellegrini et al. (1997).

Predicted cheese yields were 23.7 ± 0.7% in MN ewes and 18.9 ± 0.6% in LC ewes, on average (equivalent to 4.2 and 5.3 kg of milk per kg of cheese, for MN and LC, respectively). No significant correlations between RCT and cheese extract were found by breed (r = 0.16 to 0.19, $P > 0.05$).

Table 3.4. Effects of shearing treatments on milk composition and milk coagulation traits at d 160 in 2 breeds of dairy ewes (data are LSM).

	Shearing treatment[1]			Mean ± SEM	Effect (*P*-value)
Breed and item	CO	SBB	S100		ST[1]
Manchega					
Total solids, %	21.04	21.42	21.55	21.33 ± 0.41	0.61
Fat, %	8.37	8.61	8.70	8.56 ± 0.27	0.62
Protein, %	7.25	7.28	7.19	7.23 ± 0.24	0.96
Lactose, %	4.61	4.62	4.72	4.65 ± 0.07	0.47
Protein/Fat ratio	0.85	0.85	0.83	0.84 ± 0.03	0.76
Cheese extract[2], %	15.49	15.88	15.89	15.75 ± 0.44	0.73
Cheese yield[3], %	23.3	23.9	23.9	23.7 ± 0.7	0.72
F45[4], mm	46.6	44.6	46.7	46.0 ± 1.5	0.53
RCT[5], min	9.23[b]	9.56[ab]	10.02[a]	9.60 ± 0.14	0.004
Lacaune					
Total solids, %	18.50	17.84	18.21	18.19 ± 0.38	0.46
Fat, %	6.93	6.31	6.43	6.57 ± 0.28	0.24
Protein, %	6.20	5.79	6.46	6.16 ± 0.23	0.13
Lactose, %	4.50	4.82	4.70	4.67 ± 0.12	0.16
Protein/Fat ratio	0.90	0.93	0.97	0.93 ± 0.03	0.35
Cheese extract[2], %	13.13	12.10	12.67	12.63 ± 0.40	0.20
Cheese yield[3], %	19.7	18.1	18.9	18.9 ± 0.6	0.20
F45[4], mm	42.5	45.4	39.8	42.6 ± 2.82	0.41
RCT[5], min	11.27[a]	11.03[a]	10.41[b]	10.87 ± 0.16	0.008

[a,b]Different letters in the same line indicate differences ($P < 0.05$) by treatment; [1]shearing treatment (CO, control; SBB, shorn before breeding; S100, shorn at d 100 of pregnancy); [2]fat + protein; [3]according to Van Slyke equation (Mullan, 2008); [4]curd firmness at 45 min; [5]rennet coagulation time.

3.5 CONCLUSIONS

The results of the present study provide evidence that pregnancy shearing (d 100) in MN and LC ewes, improved the body fatness during late-pregnancy which increased glycemia at lambing in the MN ewes, but only numerically in the LC when compared to control. These results support an improved metabolic status to prioritize fetus and mammary gland development during late-pregnancy. Despite this, not marked carry-over effects were observed during the following suckling and milking periods of lactation. Thus, no effects on lamb weight,

milk yield and composition (except milk protein and casein during suckling) were detected in MN ewes, although milk yield tended to increase, but not composition in the LC ewes during the machine milking period. Milk coagulation traits were differentially affected by pregnancy shearing, the coagulation of MN ewe's milk was delayed whereas that of LC ewes was accelerated.

In conclusion, the use of pregnancy shearing is recommended as a husbandry practice to improve the welfare of dairy ewes because it alleviates the impact of heat stress during summer and reduces the loss of body reserves until lambing. Moreover, no negative effects on performances are expected in the subsequent lactation (e.g., colostrum composition, milk yield and composition, coagulation traits, birthweight, and growth of the lambs) and an increased milk yield may be also expected in high yielding ewes.

CHAPTER 4

Metabolic and productive characteristics of sensitive and heat tolerant dairy sheep phenotypes

CHAPTER 4

Metabolic and productive characteristics of sensitive and heat tolerant dairy sheep phenotypes

4.1 ABSTRACT

With the aim of studying the lactational effects of heat stress (HS) on Manchega dairy ewes, 2 experiments were carried out under climatic chamber conditions. First, 24 ewes in late lactation (1.04 ± 0.04 kg/d, 158 ± 5 DIM and 68.6 ± 1.2 kg BW) were submitted to a short-term (90 min) heat challenge (35.6ºC at 43% humidity; THI_{NRC} = 85). Rectal temperature (RT) and respiratory rate (RR) were recorded before and after the challenge, and the ewes classed by the change ratio (CR = after/before). CR values were normally distributed (5.15 ± 0.22) and a subset of 10 ewes differing in CR phenotype (Tolerant: T, 4.61 ± 0.24, n = 5; Sensitive: S, 5.69 ± 0.14, n = 5; $P < 0.01$) were selected. Second, the 10 chosen ewes were used in a crossover design of 2 periods (3 wk each) and 2 climatic conditions: 1) thermo-neutral (TN; 15 to 20°C day-night), and 2) HS (day, 37°C; night, 30°C). The THI_{NRC} values (day-night) were: TN = 65-63 and HS = 87-79. The RT, RR, milk yield, and feed and water intakes were recorded daily, whereas milk and blood samples were collected and analyzed weekly. At the end of each period, a glucose tolerance test (GTT) was performed, in which blood samples were collected at 10 time-points (min –15 to 120) and analyzed for glucose and insulin. The HS ewes increased RT (0.54ºC), RR (126 and 227% at a.m. and p.m., respectively) and water intake (35%), whereas they decreased feed intake (20%) compared to TN ewes. Milk yield (0.63 ± 0.05 kg/d) did not vary, but milk fat and milk protein contents decreased by 14 and 17%, respectively in HS ewes. The TN and HS ewes had similar blood glucose, insulin, but HS had greater NEFA (74%), prolactin (415%) and creatinine (10%) than TN ewes. Comparing T and S phenotypes, no differences were detected in feed and water intakes, milk yield and composition, or blood metabolites. However, T vs. S ewes had different magnitude of increase in RT (0.47 vs. 0.61ºC at p.m.) with lower increment in water consumption (24 vs. 45%) in response to HS. Blood metabolites did not vary between T and S phenotypes. In conclusion, Manchega dairy ewes in late lactation were relatively tolerant to HS conditions, with some differences between heat tolerant and sensitive phenotypes.

4.2 INTRODUCTION

Dairy sheep farming around the world is largely concentrated in the Mediterranean area, generally characterized by warm to hot, dry summers and mild to cool, wet winters, with specific regional characteristics (Ramón et al., 2016). Livestock activity has a significant economic

importance in Spain, where the Manchega breed, mainly located in the Autonomous Community of Castilla-La Mancha, is devoted to the production of Manchego cheese under PDO (Protected Designation of Origin) conditions (Gallego et al., 2016). Castilla-La Mancha, which derive from Arabic "*al mansha"* means "dry land" (Sánchez, 1994), suffers hot and dry periods in summer (3-5 mo), where the animals are exposed to significant heat stress (HS). Additionally, according to the predictions of climate change, the Mediterranean basin is considered one of the regions where higher temperature increases are expected (Segnalini et al., 2013).

High ambient temperature, humidity, and radiation are climatic factors that impose strain on animals (Silanikove, 2000). Additionally, heat waves (frequency and intensity) affect animal welfare and performance (Gaughan et al, 2009). Since animals adopt a series of responses at different levels trying to acclimate to the extreme environmental conditions, this process requires additional energy that would have been devoted to production and reproduction (Lu, 1989; Indu et al., 2014; Collier et al., 2018).

The assessment of animal response to HS (e.g., thermophysiological traits, productive performance, metabolic indicators, gene expression) is essential to understand the implicated underlying mechanisms and contribute to the implementation of possible alleviation strategies. These strategies include genetic improvement for thermotolerance that requires identification of thermotolerant animals, which theoretically are those that maintain homeothermy as well as productive and reproductive levels under high environmental heat load (Carabaño et al., 2019). However, finding the balance between production and thermotolerance is complex given the existing evidence of the antagonism between milk yield and heat tolerance in dairy animals (West, 2003; Finocchiaro et al., 2005). Usually, high productive animals have greater metabolic rate and consequently greater sensitivity to HS.

As far as we know, studies assessing the response of Manchega dairy ewes to HS under controlled conditions are lacking. We hypothesized that HS would modify productive and metabolic status of dairy Manchega ewes. Additionally, given the high variability between individual animals, response to HS could vary according to the phenotype (i.e., sensitive vs. tolerant). The objectives of the current work were to: 1) assess the productive and metabolic responses of Manchega dairy ewes when exposed to HS, and 2) evaluate whether these

responses would differ between tolerant and sensitive phenotypes previously classified under controlled acute heat tolerance test.

4.3 MATERIALS AND METHODS

The experimental procedures were approved by the Ethics Committee of Animal and Human Experimentation of the Universitat Autònoma de Barcelona, UAB (Ref. 3142) and agreed with the codes of recommendations for the welfare of livestock of the Ministry of Agriculture, Food and Environment of Spain.

4.3.1 Heat Tolerance Test

Twenty-four multiparous Manchega dairy ewes in late lactation (1.04 ± 0.04 kg/d, 158 ± 5 DIM and 68.6 ± 1.2 kg BW) with healthy and symmetrical udders, from the herd of the Universitat Autònoma de Barcelona (UAB), were enrolled.

The heat tolerance test was carried out in a 4 × 6 × 2.3 m climatic chamber (Euroshield Oy, ETS Lindgren-Euroshield Oy, Eura, FI) equipped with temperature and ventilation (Duelectron Controls Ibérica, Barcelona, ES), humidity (20 to 90% RH) (Carel Controls Ibérica, Barcelona, Spain), and light control systems. Animals were provided with white light for 12 h (0800 to 2000, ≈500 lux) and red light for 12 h (2000 to 0800, ≈10 lux) by led-lamps. Ewes were evaluated for their thermophysiological response in terms of rectal temperature (RT) and respiratory rate (RR) when subjected to a heat stress test according to the methodology described by Elhadi and Caja (2018).

Rectal temperature was recorded using a digital clinical thermometer (AccuVet, Cei Technology, Taoyuan City, TW; range, 32 to 45°C; accuracy, ± 0.1°C). Respiratory rate was visually measured by 2 trained operators without disturbing the animals, by counting breaths during 15 s and expressed as breaths/min.

The heat tolerance test was performed in 2 consecutive days, during which the ewes were taken to the chamber after the afternoon milking (1800 h) and housed in sawdust bedded pens (1.75 m × 0.90 m; n = 2-3 animals / pen). Ewes were subjected to thermoneutral conditions (TN; 20ºC and 45% HR; THI = 65) overnight (Figure 4.1) with water and feed available *ad libitum*. The next morning (0900 h), water and feed were removed, and the basal thermophysiological variables were measured.

Thereafter, the chamber temperature was gradually raised during 1 h (+ 0.3ºC per min), keeping the humidity constant to achieve heat stress conditions (HS; 37ºC and 45% RH, THI = 85), and the thermophysiological measurements were repeated after 90 min in these conditions.

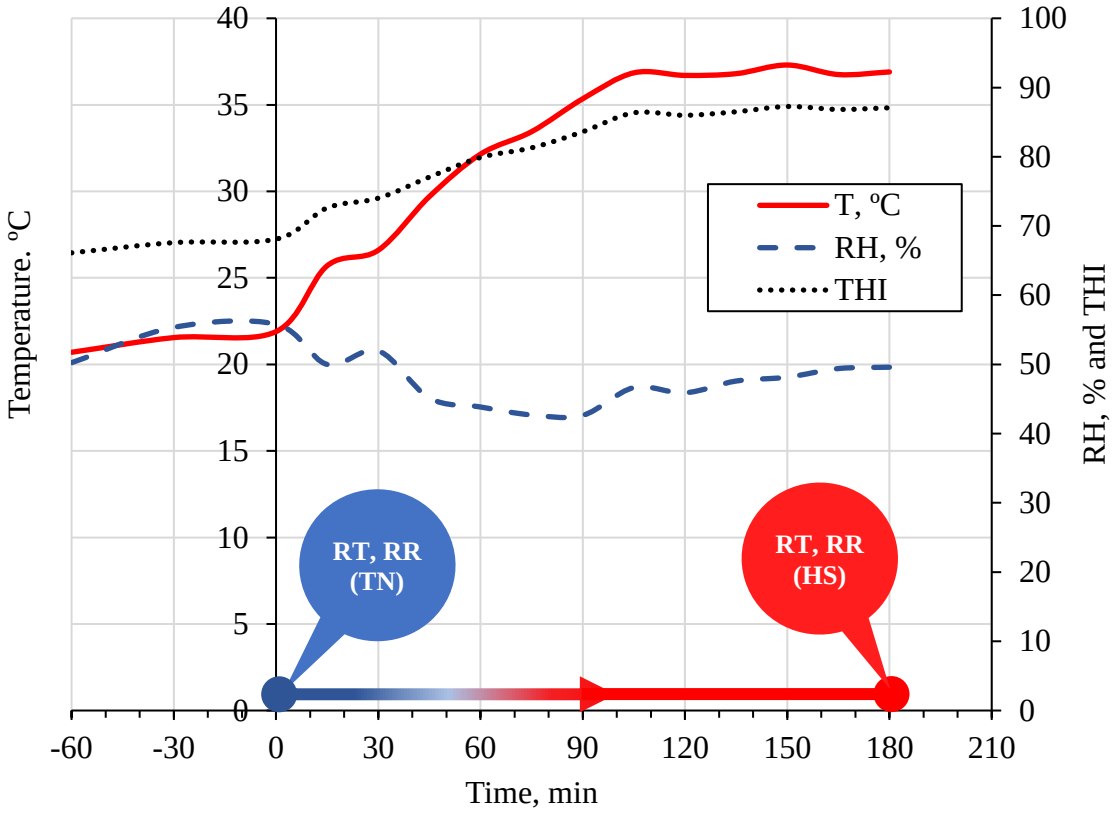


Figure 4.1. Environmental conditions of heat tolerance test. Rectal temperature (RT) and respiratory rate (RR) were measured before (blue) and after 90 min of heat challenge (red).

Ewes were classified as heat-tolerant or -sensitive according to the change ratio (CR) value obtained with the equation cited by El-Zarei et al., (2019) as follows:

$$CR = \left(\frac{newly\ recorded\ RT\ value}{basal\ RT\ value}\right) + \left(\frac{newly\ recorded\ RR\ value}{basal\ RR\ value}\right)$$

Accordingly, a CR value close to 2 means that the animal is more heat-tolerant.

4.3.2 Effect of Heat Stress on Performances

4.3.2.1 Animals, Management Conditions, and Treatments

A total of 10 ewes (1.14 ± 0.06 kg/d, 153 ± 8 DIM, and 67.5 ± 1.2 kg BW) were chosen from the heat tolerance test to form 2 groups differing in their CR values being tolerant (T; n = 5) or sensitive (S; n = 5). The experimental design was cross-over, with 2 groups of 5 ewes each subjected to 2 treatments (TN and HS) during 2 periods of 3 weeks each.

Ewes were allowed 1 week as an adaptation to the experimental conditions at the beginning of each period. Additionally, there was 2 weeks washing out between periods 1 and 2. In the first period, 2 T and 3 S ewes were subjected to TN, whereas 3 T and 2 S ewes were under HS. In the second period, ewes were switched to the opposite treatment. Throughout the experiment, ewes were maintained in individual pens (1.75 × 0.9 m).

Conditions of the TN were: 15 to 20°C and 50 to 60% relative humidity throughout the day; THI = 63 to 65. For the HS treatment, the temperature was increased gradually in 3 consecutive days, from 20 to 25 °C on the first day, from 25 to 30°C on the second day and from 30 to 37°C on the third day. Thereafter, temperature was kept at 37°C from 0900 to 2100 h and 30°C from 2100 to 0900 h. Relative humidity was kept at 50 ± 5%. These HS conditions resulted in THI = 87 and 79 for the day and night, respectively.

For both TN and HS ewes dark-light (12-12 h) were maintained constant throughout the experiment. The THI values were calculated according to NRC (1971):

$$THI = (1.8 \times Tdb + 32) - [(0.55 - 0.0055 \times RH) \times (1.8 \times Tdb - 26.8)]$$

where Tdb is the dry bulb temperature (ºC) and RH is the relative humidity (%).

Environmental data of temperature (T, °C) and relative humidity (RH, %) were checked twice a-day (0800 and 1700 h) using a dry- and wet-bulb thermo-psychrometer (Herter, Barcelona, ES; accuracy ± 0.5°C).

Ewes were milked twice daily (0800 and 1700 h) with a portable milking machine (Westfalia-Separator Iberica; Granollers, ES), set at 42 kPa, 120 pulses/min and 66% pulsation ratio, provided with a collecting jar (5 L). Milking routine included cluster attachment without udder

preparation or teat cleaning, machine milking before cluster removal, and teat dipping in an iodine solution (P3-ioshield; Ecolab Hispano-Portuguesa S. L., Barcelona, ES).

Feed was offered *ad libitum* once-a-day after milking at 20% of the previous day refusal, as a total mixed ration (forage: concentrate, 40:60%), formulated according to INRA (2018). The diet consisted of (as fed) alfalfa hay 60%, soybean oil 2%, oat 4.5%, barley grain 37%, rapeseed flour 5%, crashed corn grain 15%, sunflower meal 2%, gluten feed 10%, molasses 2%, soybean husk 15%, soybeanmeal-44 5%, salt 0.5%, calcium carbonate 0.5%, dicalcium phosphate 1%, and vitamin-mineral corrector 0.3%. Mineral blocks (Multi-Block;AgràriaComarcal del Vallès, Llerona, ES) were freely available to each ewe (Na 16%, Ca 12 %, Mg 2.2 %, P 5.5%, Zn 2000 mg/kg, Mn 1000 mg/kg, Se 15 mg/kg, Fe 40 mg/kg, I 60 mg/kg, Co 40 mg/kg, vitamins: vit A 120000 UI/kg, vit E 120mg/kg). Clean water was freely available at ambient temperature.

4.3.2.2. Sample Collection, Measurements, and Analyses

Rectal temperature and respiration rate. Rectal temperatures and respiratory rates were daily recorded at 0800 and 1700 h as indicated in the heat tolerance test.

Feed intake and water consumption. Feed intake and water consumption were daily recorded throughout the experiment by a digital scale (Sartorius; Göttingen, DE; accuracy, 30 g). Feed samples (offered and refused) were collected at the beginning and the end of each experimental period, ground through a 1 mm stainless steel screen, and analyzed for DM, ADF, NDF, and ash contents according to the analytical standard methods (AOAC International, 2003). The Dumas method (AOAC International, 2003) with a Leco analyzer (Leco Corporation, St. Joseph, MI) was used for N determination, and CP was calculated as percentage of N × 6.25. Chemical composition and nutritive value of the ration are shown in Table 4.1.

Milk yield and milk composition. Milk yield (kg/d) of the individual ewes was measured daily at each milking throughout the experiment by the same digital scale used for feed intake and water consumption measurements.

Milk composition was evaluated weekly by collecting approximately 100 mL of composite milk from the a.m. and p.m. milkings. Milk samples were preserved with an antimicrobial tablet (bronopol, Broad Spectrum Microtabs II; D & F Control Systems Inc., San Ramon, CA) and kept at 4°C until analysis. Milk samples were analyzed for the contents of total solids (TS), fat,

crude protein (CP; N × 6.38), lactose, and 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 ewe milk.

Table 4.1. Chemical composition and nutritive value of the ration used for Manchega dairy ewes.

Item	Total mixed ration
Component %	
Dry matter	87.0
Organic matter	90.5
Crude protein	16.3
Ether extract	2.22
Neutral detergent fiber	35.4
Acid detergent fiber	24.3
Acid detergent lignin	3.71
Nutritive value[1]	
UEm, [2]/kg	1.15
UFL,[3] /kg	0.81
NEL, Mcal/kg	1.43
PDIE,[4] g/kg	105
PDIA[5], g/kg	77.3
RPB,[6] g/kg	22.6
Ca_{abs}, g/kg	2.49
P_{abs}, g/kg	4.63

[1] Calculated according to the Institut National de la Recherche Agronomique (INRA, 2018). [2] Fill units for dairy ewes (1 UEL = 1 kg DM of reference grass). [3]Forage unit for lactation (1 UFL = 1.76 Mcal of NEL).[4] Net energy for lactation[5] Protein digestible in the intestine from dietary and microbial origin. [6] Protein digestible in the intestine from dietary origin.[7] Rumen protein balance.

Body weight. Body weight (BW) of each ewe was recorded at the beginning and the end of each period. Weighing was performed using a mobile weighing scale (WA200, Meier-Brakenberg, Brakenberg, DE).

***Blood measurements*.** Blood samples were taken at d 0, 7, 14, and 19 from the jugular vein into 10-mL vacutainers with spray-coated K2-EDTA (BD Diagnostics, Franklin Lakes, NJ) before the morning feeding. Plasma was obtained by centrifugation of whole blood for 15 min at 2000 × *g* and 4°C, and stored at −20°C until the analysis of glucose, urea, NEFA, insulin, prolactin, and creatinine.

The NEFA were determined by colorimetric enzymatic test ACS-ACOD method using a commercial kit (Wako Chemicals, Neuss, Germany). Insulin and prolactin were measured by sandwich-type ELISA using commercial kits (Mercodia Ovine Insulin ELISA, Mercodia, Switzerland; PRL-ELISA, DIASource Immunoassays S.A., Belgium; Ireland). Glucose was determined by hexokinase method (OSR 6121, Reagent System Olympus, Beckman Coulter, Ireland), whereas urea by Urea U.V. kinetic method and creatinine by Jaffe method.

Glucose tolerance test. Glucose tolerance tests (GTT) were conducted in the last week of each period at 1000 h. Glucose (0.25 g/kg BW) solutions were administrated via a jugular catheter. Blood samples were collected at −15, 0, 5, 10, 20, 30, 45, 60, 90, and 120 min relative to glucose infusion. Blood samples were collected by syringe into 4-mL K2-EDTA spray-coated vacutainers and were immediately placed on ice. After centrifugation of whole blood for 15 min at 2000 × g and 4°C, plasma was divided into different aliquots and stored at −20°C for subsequent analysis of plasma insulin and glucose concentrations as previously indicated in weekly blood samples.

To assess the response to the GTT, the following variables were calculated for blood glucose and insulin: basal concentration, peak concentration, clearance rate, and area under curve (AUC) at 60 and 120 min. The AUC between times was calculated using the trapezoidal method, in which glucose or insulin concentrations were calculated by subtracting the actual value from the baseline value. Glucose clearance rate (GLU-CR) and insulin clearance rate (INS-CR) during the GTT were calculated according to Kerestes et al. (2009):

$$GLU - CR\ (\%/min) = \frac{ln[GLU_{\text{peak}}] - ln[GLU_{60}]}{60 - \text{t}_{\text{peak}}} \times 100$$

$$INS - CR\ (\%/min) = \frac{ln[INS_{\text{peak}}] - ln[INS_{60}]}{60 - \text{t}_{\text{peak}}} \times 100$$

Additionally, a quantitative insulin sensitivity check index (QUICKI) was calculated according to Katz et al. (2000):

$$QUICKI = \frac{1}{\log \text{insulin}(\mu U/mL) + \log \text{glucose}\ (mg/dL)}$$

4.3.3 Statistical Analyses

Data were analyzed by the PROC MIXED for repeated measurements of SAS (SAS v. 9.2 SAS Institute Inc., Cary, NC, USA). The statistical mixed model contained the fixed effects of the temperature (TN and HS), heat tolerance phenotype (T and S), period (1 and 2), experimental day (1 to 19), the interactions between the fixed effects, the random effects of the animal, and the residual error. The model considered the possible carryover effects of previous HS periods through the temperature × period interaction. For the data of rectal temperature and respiratory rate measured at 0800 and 1700 h, a fixed factor of the hour of day was added to the model. For the data of BW change and GTT responses, the PROC MIXED was used without repeated measurements, and consequently the day effect was removed from the model.

Differences between least square means were determined with the PDIFF test of SAS. Significance was declared at $P < 0.05$ and tendency at $P < 0.10$ unless otherwise indicated.

4.4 RESULTS AND DISCUSSION

4.4.1. Heat Tolerance Challenge

The impact of exposing the 24 multiparous Manchega dairy ewes to a short-term heat challenge was assessed by using the CR of thermophysiological traits (RT and RR) as indicated in the previous section.

The values of CR in the current study varied between 3.72 and 6.90 (Figure 4.1). The CR values were normally distributed and averaged 5.15 ± 0.22. Maintaining homeothermy under HS conditions depends on the ability to balance between thermogenesis and heat dissipation, and there is a high variation in this ability between animals. Sánchez-Molano et al. (2019) reported that individual animals differ significantly in their response to changing ambient temperature and THI. Additionally, animals with different resilience to weather change have different genetic variance and heritability estimates. Those authors found that heritability (h^2)

estimates for resilient phenotypes ranged from 0.09 to 0.11, which is relatively low, but may allow for selective breeding for those resilient animals to weather changes.

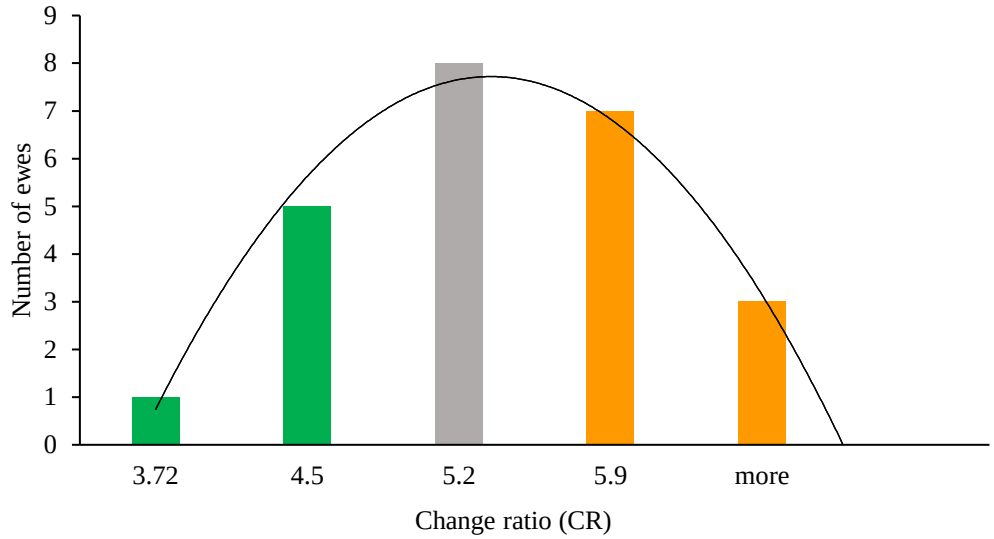


Figure 4.1. Distribution of the thermophysiological traits change ratio (CR) of Manchega dairy ewes (n = 24) submitted to a short-term heat challenge.

Several thermophysiological traits have been used to identify heat tolerant animals, including RT, RR, and heart rate. In the current study, we used the CR index that considers the magnitude of change in RT and RR when the animal is exposed to acute heat shock treatment since these thermophysiological traits are considered good animal-based indicators for heat tolerance (Finocchiaro et al., 2005; Salama et al., 2016).

The wide range of CR detected in the current study, using only 24 ewes, confirms the known variability in the response to HS, and might allow the selection for heat-tolerant animals. However, to be successful, the selection for these heat-tolerant animals should not result in lower performances, as high productive animals have greater metabolic rates and may suffer HS to a greater extent (West, 2003). In fact, Sánchez-Molano et al. (2019) detected that some resilience traits have a significant unfavorable genetic correlation with animal performances.

Keeping in mind what mentioned above, we carried out an experiment to test whether the tolerance to HS could be related to animal performances and metabolism. Therefore, a subset of 10 ewes differing ($P < 0.01$) in CR as heat-tolerant (T; CR = 4.61 ± 0.24, n = 5) and heat-

sensitive (S; CR = 5.69 ± 0.14, n = 5) were enrolled in the second experimental phase, during which they were submitted to HS conditions for longer time.

4.4.2 Effect of Heat Stress on Thermophysiological Traits

4.4.2.1 Heat Stress vs. Thermoneutral

Rectal temperature. Rectal temperatures tended to increase ($P < 0.10$) from 0800 to 1700 h regardless the treatment, in accordance with the expected changes by the circadian biphasic rhythm and temperature differences (morning vs. afternoon) reported in sheep (Mohr and Krzywanek 1990; Monty et al, 1991) and goats (Hamzaoui et al., 2013). Figure 4.2 shows the RT variation throughout the experimental days by treatment and hour of measurement.

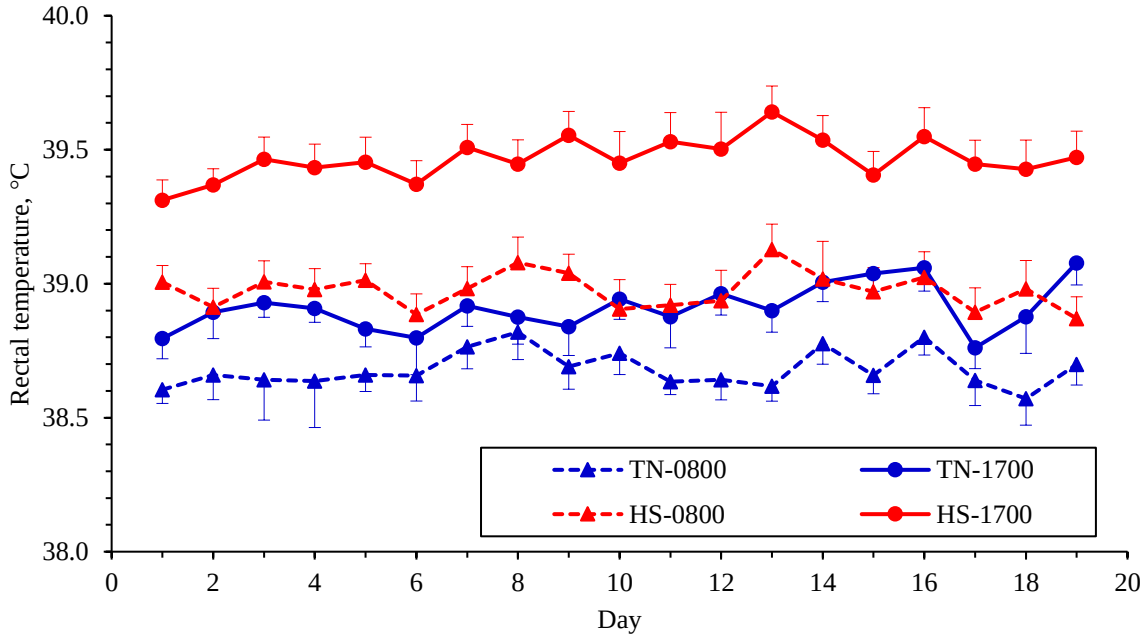


Figure 4.2. Daily rectal temperatures in the morning and in the afternoon of Manchega dairy ewes in late lactation under thermoneutral (TN, n = 10) or heat stress (HS, n = 10) conditions. Values are means ± SEM.

On average and compared with TN, RT of ewes under HS conditions increased by 0.54°C ($P < 0.001$) at 0800 and 1700 h as shown in Table 4.2. The significant rise of 1.4% in RT was within the reported range for sheep (1 to 3%) exposed to controlled HS conditions (Stephenson et al., 1980; Al-Haidary, 2004; Mehaba et al., 2021).

Table 4.2. Average rectal temperature (RT) and respiration rate (RR) in the morning and in the afternoon of Manchega dairy ewes in late lactation under thermoneutral (TN, n = 10) or heat stress (HS, n = 10) conditions.

	Treatment			HS vs. TN	Effect (*P*-value)		
Item	TN	HS	±SEM	change, %	Treatment	Hour	T×H[1]
RT, ºC							
0800	38.67	39.21	0.11	1.4	0.001	0.07	0.99
1700	38.90	39.44	0.11	1.4	0.001		
RR, bpm							
0800	39	88	3	126	0.001	0.001	0.001
1700	41	134	3	227	0.001		

[1]Treatment × hour interaction.

Also, the RT of HS ewes at 0800 h was only 0.3ºC greater than the RT of TN ewes at 1700, which might indicate that, during the lower temperature of the night (30ºC), HS ewes were able to approach normal body temperatures.

Respiratory rate. No differences in RR values were detected throughout the day in the TN ewes (Table 4.2 and Figure 4.3) that averaged 40 bpm, agreeing with the reported value in Corriedale sheep under THI of 63 (Kitajima et al., 2021). On the contrary, when comparing HS vs. TN, ewes increased their RR by 126 and 227% at 0800 and 1700 h, respectively (Table 4.2) indicating, as expected, a greater response in the afternoon when ewes suffered the highest heat load. Similarly, Mehaba et al., (2021) reported that heat-stressed Lacaune ewes (THI 75 to 83) increased RR by 214%.

Figure 4.3 shows how respiratory rate values peaked in HS ewes at the end of the first week and then gradually decreased throughout the experiment, which indicates a fast partial acclimatization to HS conditions, as described by Hamzaoui et al. (2013) in heat-stressed dairy goats. This pattern reflects the dynamic nature of animal responses, where the acute ones involved marked RR increase as the main evaporative mechanism in sheep during periods of high heat load (Hales and Brown, 1974), while prolactin plasma values were maintained high throughout the experiment (see hereafter; Figure 4.13) acting as a supportive dissipating mechanism.

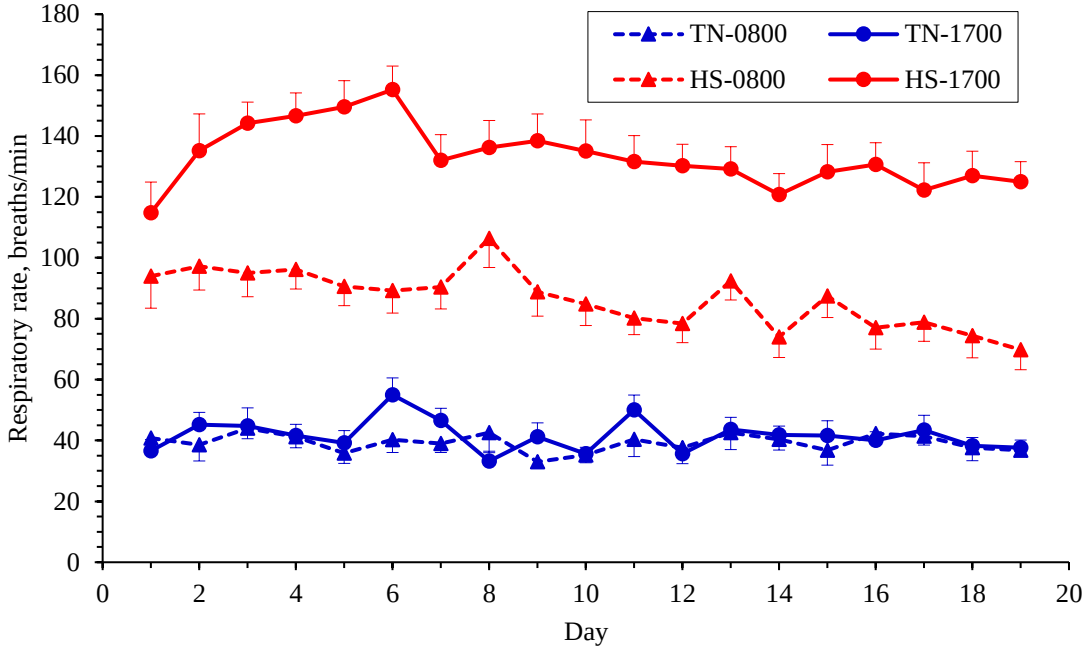


Figure 4.3. Daily respiratory rate in the morning and in the afternoon of Manchega dairy ewes in late lactation under thermoneutral (TN, n = 10) or heat stress (HS, n = 10) conditions. Values are means ± SEM.

These results show that ewes activated evaporative mechanism to dissipate extra heat load via respiratory tract when exposed to high THI (79 to 87), indicating that they undergone HS, and that the response, in terms of RR, was stronger during the first week. In this sense, the process of acclimatization takes weeks (acute and chronic) and varies according to the magnitude of the heat challenge and species (Collier et al., 2006; Renaudeau et al., 2012).

4.4.2.2 Tolerant vs. Sensitive Phenotypes

Rectal Temperature. Comparing the daily variations of RT in those ewes classified as tolerant (T) or sensitive (S) according to the heat tolerance test, a similar evolution pattern was observed during the day as shown in Figure 4.4 and Table 4.3.

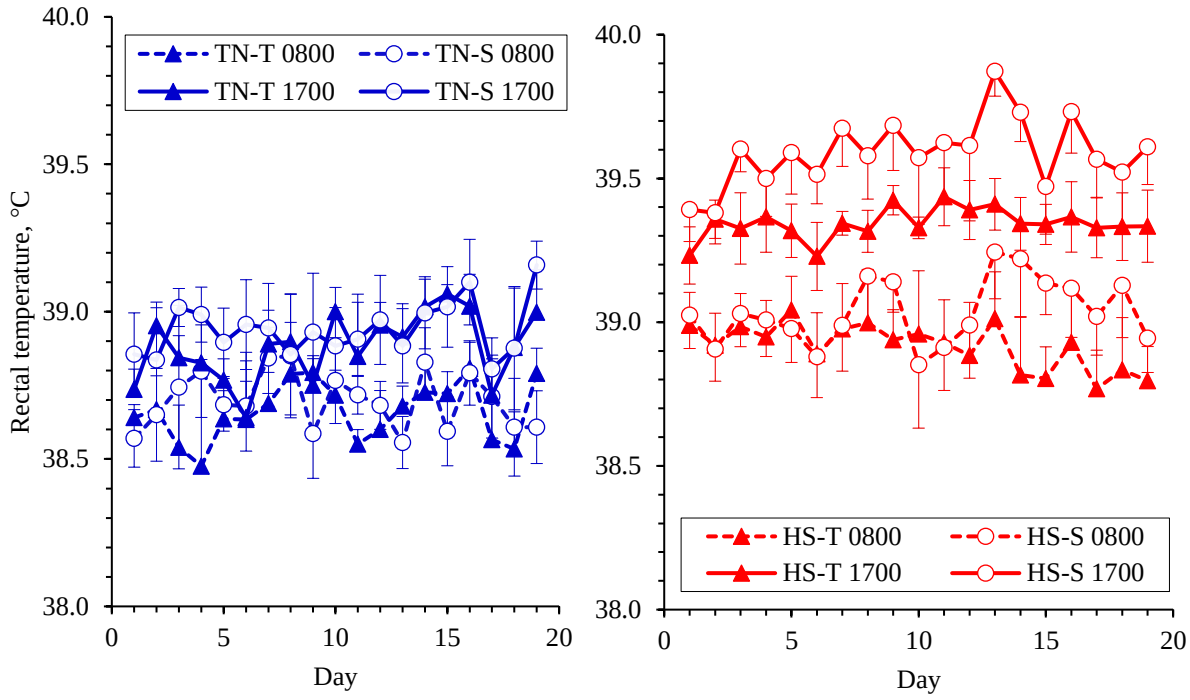


Figure 4.4. Daily rectal temperatures of Manchega dairy ewes of different heat tolerance phenotypes (tolerant, T; sensitive, S) in late lactation under thermoneutral (TN, n = 10) or heat stress (HS, n = 10) conditions. Values are means ± SEM.

Shifting from TN to HS, T and S ewes tended to increase ($P < 0.10$) RT by +0.35 and +0.80ºC, respectively, at 0800 h, with no significant temperature × phenotype interaction. In the afternoon, when the heat load was at its maximal level, there was a treatment × phenotype interaction ($P < 0.05$), where S ewes experienced greater (+0.61ºC) RT increment than T ewes (+0.47ºC) with regard to their values in TN. As shown in Figure 4.4., S ewes experienced constantly greater RT at 1700 h throughout the experimental days compared to T ewes. These results support the higher adaptive ability (lower variation in RT) of tolerant ewes than sensitive ones when exposed to maximal HS conditions.

Table 4.3. Average rectal temperature (RT) and respiratory rate (RR) in the morning and in the afternoon of Manchega dairy ewes of different heat tolerance phenotypes (tolerant, A; sensitive, B) in mid-late lactation, under thermoneutral (TN, n = 10) or heat stress (HS, n = 10) conditions.

	TN		HS			Effect (*P*-value)		
Item	T	S	T	S	±SEM	Treatment	Phenotype	T×Ph[1]
RT, ºC								
0800	38.59	38.70	38.94	39.50	0.33	0.07	0.28	0.47
1700	38.88	38.96	39.35	39.57	0.08	0.001	0.14	0.015
			+0.47	***+0.61***				
RR, bpm								
0800	37	41	86	88	4	0.001	0.53	0.72
1700	42	42	131	137	4	0.001	0.57	0.20

[1]Treatment × phenotype interaction.

Respiratory Rate. Changes throughout the day in the RR of both heat tolerance phenotypes are shown in Figure 4.5 and averages in Table 4.3. Phenotype had no effect on RR, and no treatment × phenotype interaction was detected. Changing from TN to HS, RR at 0800 h increased similarly in T (+49 bpm) and S (+47 bpm) ewes. At 1700 h, the increment was +89 and +95 bpm in T and S ewes, respectively. The S ewes had numerically greater respiratory rate (+6 bpm) and constantly experienced greater RT at 1700 h (Figure 4.4) than T ewes, which implies greater effort and energy to dissipate heat load. Similarly, heat resistant Turpan black ewes had less RR (−10%) and RT (−0.8%) than the sensitive Kazakh ewes during summer (Haire et al, 2022).

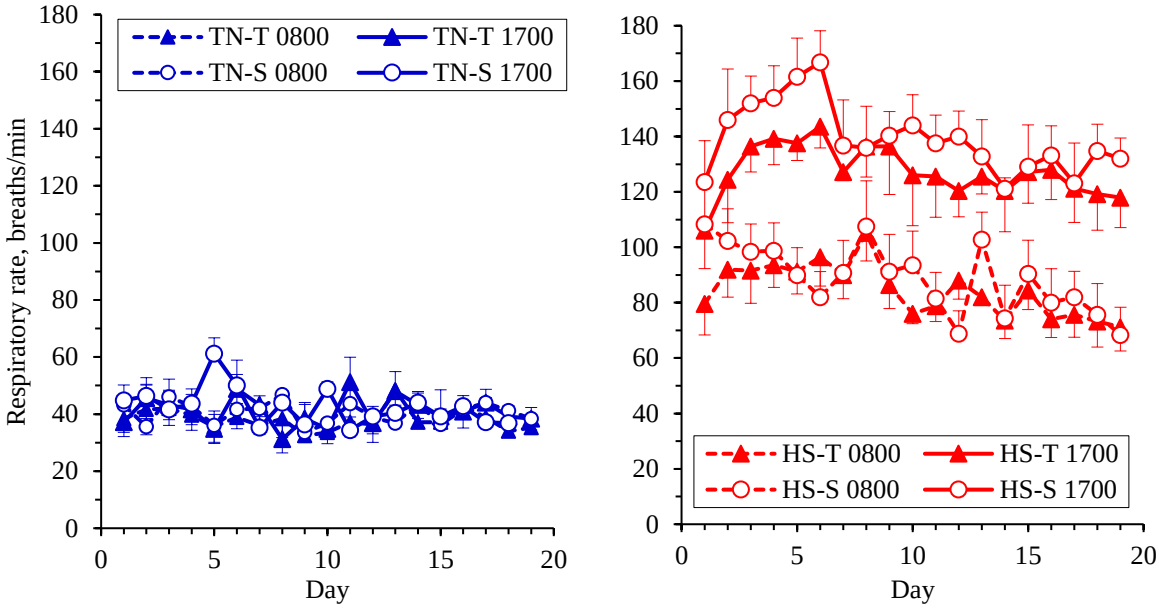


Figure 4.5. Daily respiratory rates in Manchega dairy ewes of different heat tolerance phenotypes (tolerant, T; sensitive, S) in late lactation under thermoneutral (TN, n = 10) or heat stress (HS, n = 10) conditions. Values are means ± SEM.

4.4.3 Effect of heat stress on lactational performances

4.4.3.1 Heat stress vs. thermoneutral

Feed Intake. As expected, HS markedly decreased DMI throughout the experiment (Figure 4.6) by 20% on average ($P < 0.001$; Table 4.4). This reduction in feed intake is a well-documented adaptive response of heat-stressed animals that contribute to decrease metabolic heat (Sejian et al., 2018; Pragna et al., 2018) given that feeding, especially in ruminants, is an important source of heat production (West, 2003). Thus, it is considered as one of the first signs of heat stress in dairy cattle (Noordhuizen and Bonnefoy, 2015). Similarly, this decline in feed intake was also reported in heat-stressed dairy goats (Hamzaoui et al., 2013; Salama et al., 2014; Contreras-Jodar et al., 2018) and sheep including Awassi, St. Croix, Karakul and Rambouillet breeds (Bhattacharya and Hussain, 1974; Monty et al., 1991), and Lacaune dairy ewes (Mehaba et al., 2021).

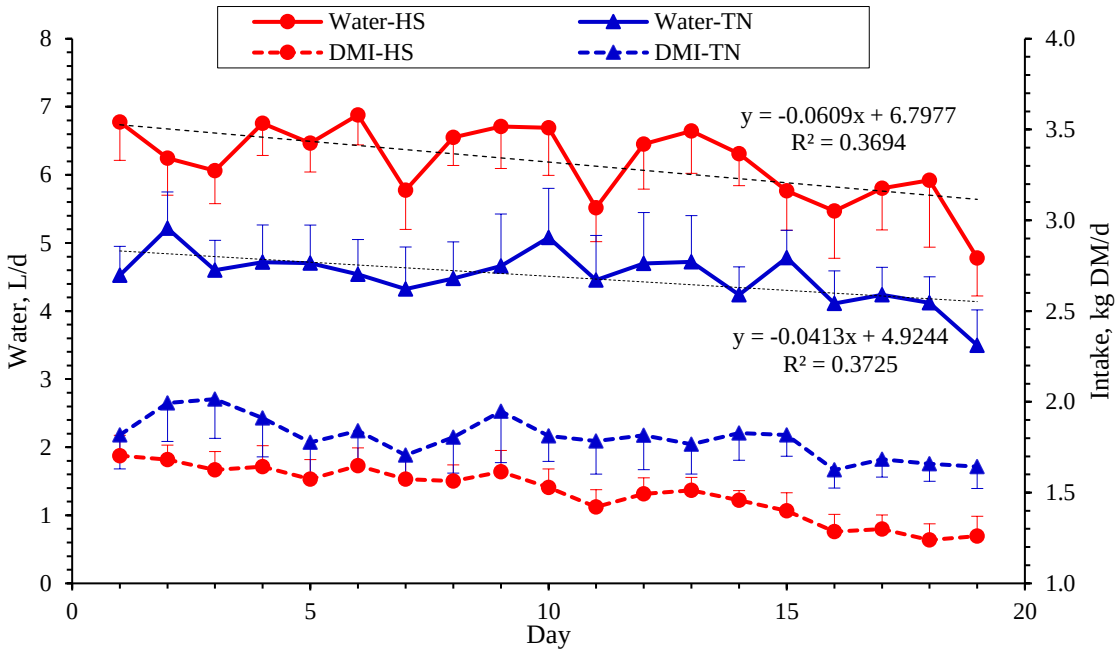


Figure 4.6. Daily dry matter intake and water consumption of Manchega dairy ewes in late lactation under thermoneutral (TN, n = 10) or heat stress (HS, n = 10) conditions. Values are means ± SEM.

Water Consumption. Ewes under HS consumed 35% more water than ewes under TN conditions ($P < 0.001$; Table 4.4). As shown in Figure 4.6, there was a gradual decrease in water consumption in both treatments as lactation advanced since milk production level is one of the factors determining water consumption (Meyer et al., 2004). Moreover, a greater decreasing slope was observed in HS vs. TN ewes (–61 vs. –41 mL/d), that was not explained by the decrease in milk yield, since milk yield decreased similarly in both treatments throughout the experiment (see later). Water to DM intake ratio in TN and HS were 2.4 and 4.1 L/kg DM, respectively, indicating increased water requirements for the mitigation of HS effects. In this regard, HS induces a marked increase in water turnover due to enhanced evaporative heat dissipation in sheep, mainly by panting and to a lesser extent by sweating (Marai et al., 2007).

Table 4.4. Feed and water intake and body weight change of Manchega dairy ewes in late lactation under thermoneutral (TN, n = 10) or heat stress (HS, n = 10) conditions throughout the experiment.

	Treatment			HS vs. TN	Effect (*P*-value)		
Item	TN	HS	±SEM	change, %	Treatment	Period	T×P[1]
BW change, kg	0.24	0.12	0.87	−50	0.92	0.99	0.19
DM intake, kg/d	1.87	1.50	0.08	−20	0.001	0.002	0.118
Water consumption, L/d	4.58	6.18	0.32	35	0.001	0.001	0.061

[1]Treatment × period interaction

Body Weight Change. No significant differences were detected in BW change between TN and HS conditions ($P > 0.05$; Table 4.4) despite the reduction in feed intake. This may be because our ewes were in late lactation, and despite the reduction in DMI, HS ewes were still in positive energy balance, and consequently did not loss BW. In this regard, Lacaune dairy ewes under HS conditions (THI = 75 to 83) lost BW because they suffered negative energy balance (Mehaba et al., 2021).

Milk Yield. On average, milk yield and ECM did not vary between TN and HS ewes ($P > 0.05$) throughout the experiment (Figure 4.7 and Table 4.5), which can be explained by the fact ewes were in late lactation. In this sense, it is documented that HS impact on milk yield is influenced by 1) stage of lactation in dairy cows (Tao et al, 2018) and dairy goats (Hamzaoui et al. 2013) with more milk yield losses at earlier stages, and 2) the species, with dairy cows being more sensitive to HS than small ruminants (Collier et al., 1981; Spiers et al., 2004).

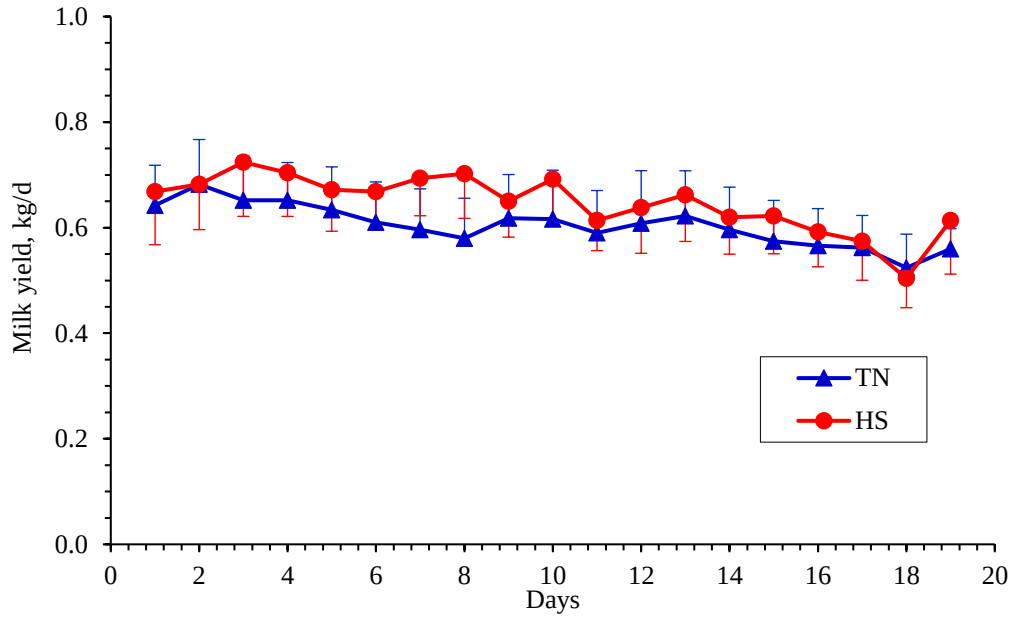


Figure 4.7. Daily milk yield of Manchega dairy ewes in late lactation under thermoneutral (TN, n = 10) or heat stress (HS, n = 10) conditions. Values are means ± SEM.

Table 4.5. Lactational performances of Manchega dairy ewes in late lactation under thermoneutral (TN, n = 10) or heat stress (HS, n = 10) conditions.

	Treatment			HS vs. TN	Effect (*P*-value)		
Item	TN	HS	±SEM	change, %	Treatment	Period	T×P[1]
Milk, kg/d							
Yield	0.61	0.65	0.05	7	0.52	0.001	0.95
ECM[2]	0.72	0.68	0.07	–6	0.51	0.001	0.96
Composition							
Fat, %	10.40	8.91	0.35	–14	0.004	0.021	0.71
Protein, %	7.64	6.35	0.26	–17	0.003	0.71	0.27
Lactose, %	4.30	4.49	0.07	4	0.09	0.08	0.50
Urea, mg/L	740	750	30	1	0.64	0.038	0.75
SCC, log/mL	4.26	4.31	0.12	1	0.69	0.001	0.69

[1]Treatment × Period interaction. [2]Energy corrected milk = Milk yield × [0.071 × (Fat, %) + 0.043 × (Total protein, %) + 0.2224], according to Bocquier et al. (1993).

Milk Composition. Milk fat and milk protein contents decreased by 14 and 17%, respectively ($P < 0.05$; Table 4.5) in ewes under HS (Figure 4.8). However, milk lactose, urea and SCC contents did not vary between treatments (Table 4.5). The decrease of milk fat and protein in the current study agrees with the known negative effects of high ambient temperatures on milk composition in dairy cows (Kadzere et al., 2002; Gao et al., 2017) and dairy goats (Hamzaoui et al., 2013; Salama et al., 2014). Regarding dairy sheep, Mehaba et al., (2021) detected similar

decrease in fat and protein (−13% and −16%, respectively) in Lacaune ewes submitted to HS conditions. Similarly, Ramón et al. (2016) reported that Manchega ewes reduce milk fat and protein yields in summer season. Both milk fat and protein decrease in heat-stressed ewes would dramatically impair the cheese yielding and coagulating properties as reported in dairy sheep (Sevi and Caroprese, 2012), goats (Abdel-Gawad et al., 2012), and cows (Bernabucci et al., 2015).

The reduced DMI and consequent decreased blood supply of milk component precursors to mammary gland, partially explains the lower milk protein in dairy cows under heat stress (Bernabucci et al., 2002b), which could indicate direct HS effects on mammary gland (Baumgard and Rhoads, 2013). Accordingly, Gao et al., (2019) detected the downregulation of genes related to milk protein synthesis and amino acid transporters in mammary tissue of heat-stressed cows. Moreover, Salama et al. (2019) reported negative impact not only on synthesis of protein (upregulation of translation inhibitor genes), but also on fat (downregulation of genes related to de novo fatty acids synthesis) in heat-stressed bovine mammary epithelial cells *in vitro*.

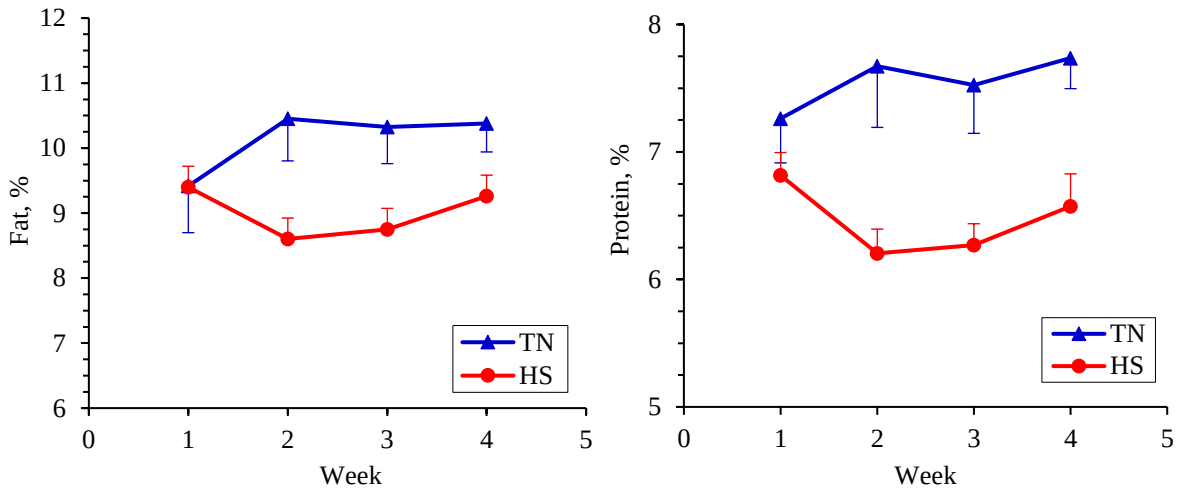


Figure 4.8. Weekly milk fat and protein contents of Manchega dairy ewes in late lactation under thermoneutral (TN, n = 10) or heat stress (HS, n = 10) conditions. Values are means ± SEM.

4.4.3.2. Tolerant vs. Sensitive Phenotypes

Feed Intake. No significant differences were detected in DMI of T and S ewes under TN and HS conditions (Figure 4.9 and Table 4.6). However, shifting from TN to HS caused a reduction in DMI by only 0.15 kg (−9%) in T ewes, whereas in S ewes the reduction was 0.41 kg (−22%) although no treatment × phenotype interaction was detected.

Water Consumption. Water consumption did not differ between the 2 phenotypes ($P = 0.81$; Table 4.6), but when comparing HS with TN values, S ewes increased their water intake by 2.0 L/d (+45%), while T ewes increased it by only 1.2 L/d (+24%). Consequently, there was significant ($P < 0.05$) treatment × phenotype interaction. This greater water consumption by S ewes can be partially explained by the fact that they breathed numerically more at 1700 h (Table 4.3), indicating an increased water turnover for thermoregulatory purpose. It seems that S ewes suffered HS at 1700 h to a greater extent compared to T ewes given that they experienced constantly greater RT (Figure 4.4).

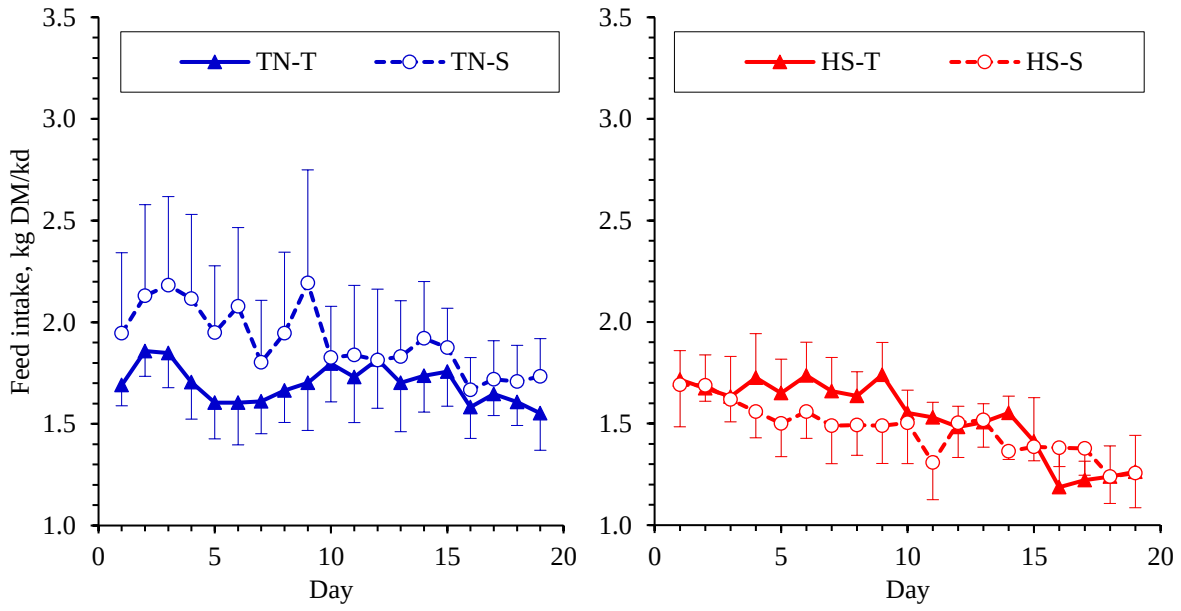


Figure 4.9. Daily feed intake of Manchega dairy ewes of different heat tolerance phenotypes (tolerant, T; sensitive, S) in late lactation under thermoneutral (TN) or heat stress (HS) conditions throughout the experiment. Values are means ± SE.

Table 4.6. Lactational performances of Manchega dairy ewes of different heat tolerance phenotypes (tolerant, T; sensitive, S) in late lactation under thermoneutral (TN) or heat stress (HS) conditions throughout the experiment.

	TN		HS			Effect (*P*-value)		
Item	T	S	T	S	±SEM	Treatment	Phenotype	T×Ph[1]
Intake								
Feed, kg DM/d	1.68	1.90	1.53	1.49	0.13	0.046	0.51	0.35
Water, L/d	4.92	4.36	6.09	6.31	0.55	0.001	0.81	0.049
Milk, kg/d			**+24%**	**+45%**				
Yield	0.59	0.63	0.60	0.66	0.08	0.37	0.62	0.78
ECM[2]	0.70	0.75	0.65	0.71	0.10	0.55	0.62	0.95
Composition								
Fat, %	10.79	9.97	9.30	8.47	0.48	0.007	0.10	0.99
Protein, %	7.62	7.69	6.39	6.37	0.37	0.001	0.95	0.90
Lactose, %	4.29	4.34	4.46	4.49	0.11	0.11	0.76	0.89
SCC, $\log_{10}$	4.18	4.25	4.54	4.20	0.12	0.16	0.20	0.06
Urea, mg/L	774	772	764	759	24	0.64	0.89	0.97
BW change, kg	1.80	−1.32	0.78	−0.54	0.66	0.92	0.08	0.48

[1]Treatment × phenotype interaction. [2]Energy corrected milk = Milk yield × [0.071 × (Fat, %) + 0.043 × (Total protein, %) + 0.2224], according to Bocquier et al. (1993).

Milk Yield and Composition. Values of milk yield and composition were similar between T and S ewes under TN and HS conditions (Table 4.6 and Figure 4.10). Our results disagree with the findings of Sánchez-Molano et al. (2019) who showed that tolerant animals suffer lesser changes in milk production when exposed to high ambient temperatures compared to sensitive animals. The absence of significant differences in the current study could be explained by the small number of animals (although the crossover design minimizes animal variability) and the stage of lactation.

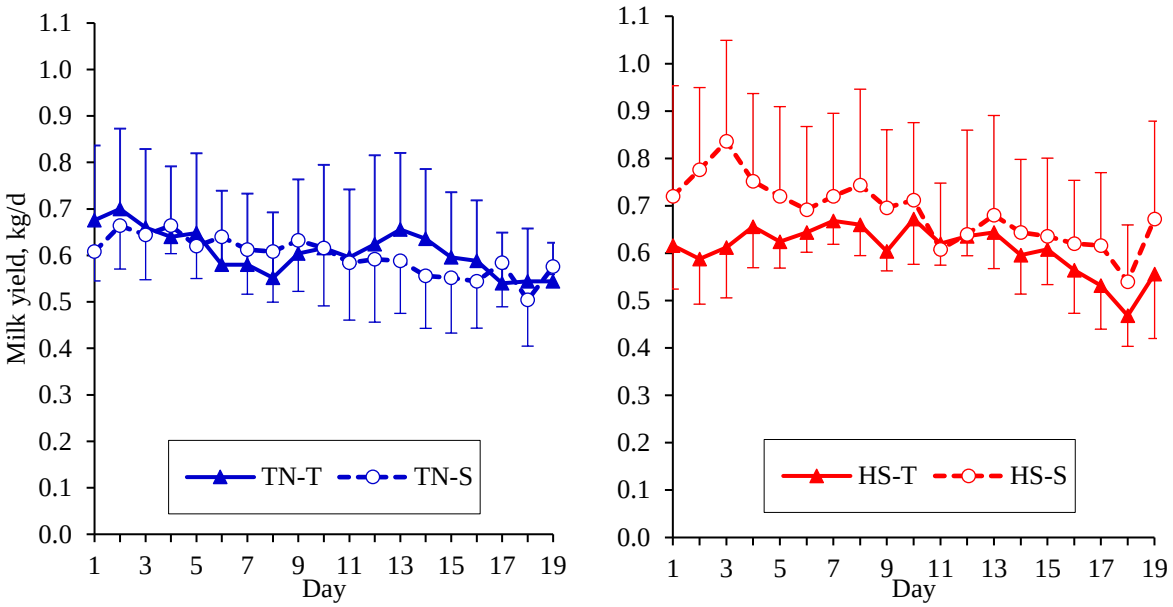


Figure 4.10. Daily milk yield of Manchega dairy ewes of different heat tolerance phenotypes (tolerant, T; sensitive, S) in late lactation under thermoneutral (TN) or heat stress (HS) conditions. Values are means ± SEM.

4.4.4. Effect of Heat Stress on Metabolic Indicators

4.4.4.1. Heat Stress vs. Thermoneutral

Results of plasma indicators are shown in Table 4.7. The basal glucose and insulin values did not differ between TN and HS ewes (Figure 4.11) as previously reported in dairy ewes (Mehaba et al., 2021). Given that insulin causes hyperthermia by direct inhibition of warm-sensitive neurons (Sanchez-Alavez et al., 2010), the lack of change in its levels in response to HS seems to be consistent with avoiding more heat production. Moreover, Achmadi et al. (1993) reported a decrease in the concentration of blood glucose without differences in plasma insulin levels in Suffolk ewes submitted to high temperatures (30°C, 70% RH). Nevertheless, Baumgard and Rhoads (2013) found that heat-stressed lactating cows experience greater blood insulin with lower glucose values compared with TN (pair feeding). The fact that plasma glucose levels did not decrease by HS might indicate that the availability of glucose (and possibly other nutrients) was not limiting for milk synthesis, given the relatively low milk production level in our ewes at late lactation.

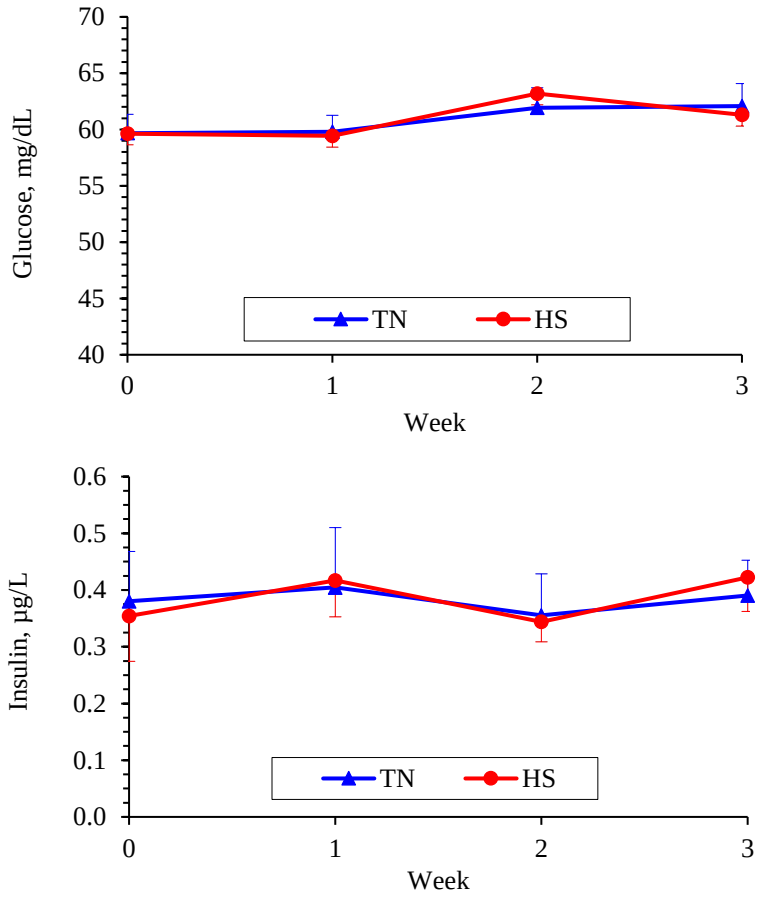


Figure 4.11. Morning values (0800 h) of plasma glucose and insulin concentrations of Manchega dairy ewes in late lactation under thermoneutral (TN, n = 10) or heat stress (HS, n = 10) conditions throughout the experiment. Values are means ± SEM.

Table 4.7. Basal plasmatic indicators of Manchega dairy ewes before the morning feeding (0800 h) under thermoneutral (TN, n = 10) and heat stress (HS, n = 10).

	Treatment			HS vs. TN	Effect (*P*-value)		
Item	TN	HS	±SEM	change, %	Treatment	Period	T×P[1]
Glucose, mg/dL	61.0	61.2	1.5	0	0.84	0.55	1.00
Insulin, µg/L	0.381	0.390	0.053	2	0.89	0.41	0.92
Prolactin, mg/dL	7.7	39.6	6.0	414	0.002	1.00	0.88
Urea, mg/L	419	414	20	6	0.85	0.73	0.39
Creatinine, mg/dL	0.830	0.910	0.031	10	0.005	0.07	0.39
NEFA, mmol/L	0.126	0.219	0.026	74	0.015	0.06	0.002

[1]Treatment × period interaction.

Despite the reduction in feed intake, HS ewes did not loss BW (Table 4.4) but increased their NEFA in plasma (P = 0.015; Table 4.7, Figure 4.12), implying body fat mobilization. This was not expected since blood insulin values (lipogenic signal) did not vary between treatments. Additionally, blood NEFA do not increase by HS in dairy cow (Baumarg and Rhoads, 2013), dairy goats (Hamzaoui et al, 2013; Mehaba et al., 2019), or Lacaune ewes (Mehaba et al., 2021). This discrepancy could stem from differences in stage of lactation, species, and breed between the current experiment and the aforementioned studies. Heat stress in known to increase the secretion of lipolytic signals (Baumgard and Rhoads, 2013), and it is possible that our Manchega secreted high concentrations of epinephrine during HS, and consequently mobilized body fat.

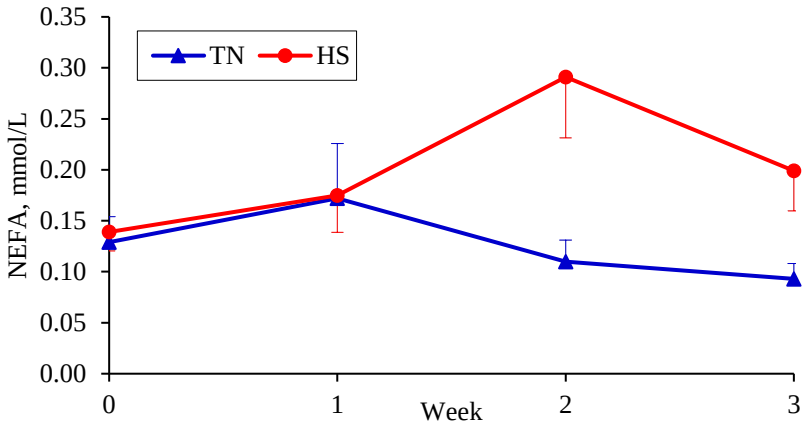


Figure 4.12. Basal plasmatic NEFA concentrations of dairy ewes under thermoneutral (TN; n = 10) or heat stress (HS; n = 10) conditions at late lactation

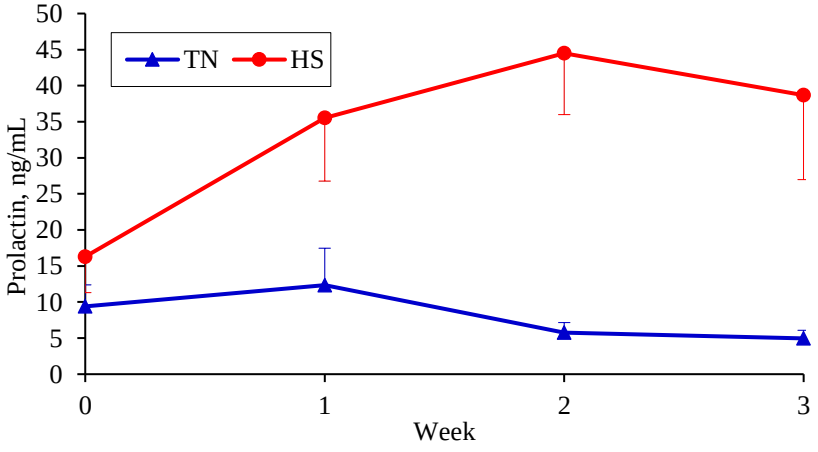
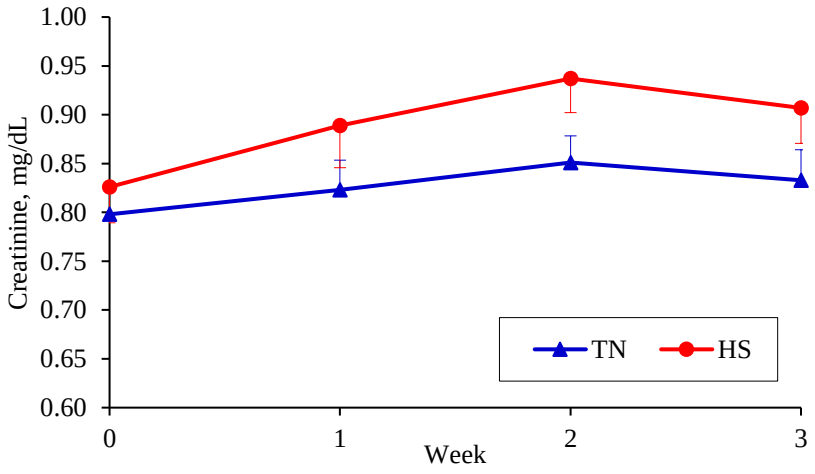


Figure 4.13. Basal plasmatic prolactin concentrations of dairy ewes under thermoneutral (TN; n = 10) or heat stress (HS; n = 10) conditions at late lactation.

On the other hand, the concentration of prolactin (PRL) increased more than 4-fold by HS ($P < 0.001$; Figure 4.13), whereas the values steadied in the TN ewes. Similar results were found by Stephenson et al. (1980), who reported that plasma PRL concentrations in thermally stressed ewes were significantly higher (+220%) than those of control ewes. Although PRL is a lactogenic hormone, milk production did not increase by HS as mentioned above, and consequently, individual PRL levels did not correlate with milk production in our data. The increase in PRL levels is involved in meeting the increased water and electrolyte demands of heat-stressed animals to maintain the extracellular fluid volume, and hence supporting heat dissipation (Alamer, 2011).

Blood creatinine levels increased ($P < 0.01$) by 10% in ewes under HS (Figure 4.14), which might indicate increased muscle degradation. Similarly, greater blood creatinine values under HS were reported in dairy ewes (+21%; Mehaba et al., 2021) and dairy goats (12%; Mehaba et al. 2019). It is possible that some glycogenic AA produced from muscle degradation were used for gluconeogenesis, resulting in keeping similar blood glucose levels between TN and HS ewes (Table 4.7).



4.14. Basal plasmatic creatinine concentrations of dairy ewes under thermoneutral (TN; n = 10) or heat stress (HS; n = 10) conditions at late lactation.

4.4.4.2. Tolerant vs. Sensitive Phenotypes

As shown in Table 4.8, heat tolerance phenotype had no effect on metabolic indicators and no treatment × phenotype was detected. Contrary to our results when comparing heat tolerant

Omani (local adapted breed) with Merino sheep subjected to HS conditions, some differences in blood chemistry were reported, including glucose changes (increases in Merino and declines in Omani). Furthermore, blood urea N is reduced in Merino and is increased in Omani (Srikandakumar et al., 2003).

Table 4.8. Basal metabolic indicators in Manchega dairy ewes of different heat tolerance phenotypes (tolerant, T; sensitive, S) in late lactation under thermoneutral (TN, n = 10) or heat stress (HS, n = 10) conditions throughout the experiment.

	TN		HS			Effect (*P*-value)		
Item	T	S	T	S	±SEM	Treatment	Ph[1]	T×Ph[2]
Glucose, mg/dL	60.7	61.4	59.4	63.0	2.0	0.85	0.41	0.11
Insulin, µg/L	0.497	0.266	0.391	0.401	0.068	0.84	0.13	0.11
Prolactin, ng/mL	8.9	9.3	41.6	37.3	8.2	0.002	0.81	0.78
Urea, mg/L	393	439	418	402	29	0.77	0.66	0.15
Creatinine, mg/dL	0.85	0.82	0.91	0.90	0.05	0.003	0.78	0.66
NEFA, mmol/L	0.109	0.141	0.195	0.248	0.033	0.011	0.23	0.76

[1]Phenotype, [2]treatment × phenotype interaction.

4.4.5. Effect of Heat Stress on Glucose Tolerance Test (GTT)

4.4.5.1. Heat Stress vs. Thermoneutral

Glucose Response to GTT. The glucose basal values averaged 61.7 ± 2.5 mg/dL and were similar in the plasma of TN and HS ewes (Figure 4.14). The values immediately rose after the glucose injection and peaked at 5 min, decreasing slowly thereafter until the last sampling time (min 120) at which the basal value was not reached. No differences were detected between TN and HS in the peak, clearance, or area under the curve (Table 4.9), although ewes under HS had numerical greater AUC values (+7 to 9%; $P = 0.17$ to 0.19) than TN ewes.

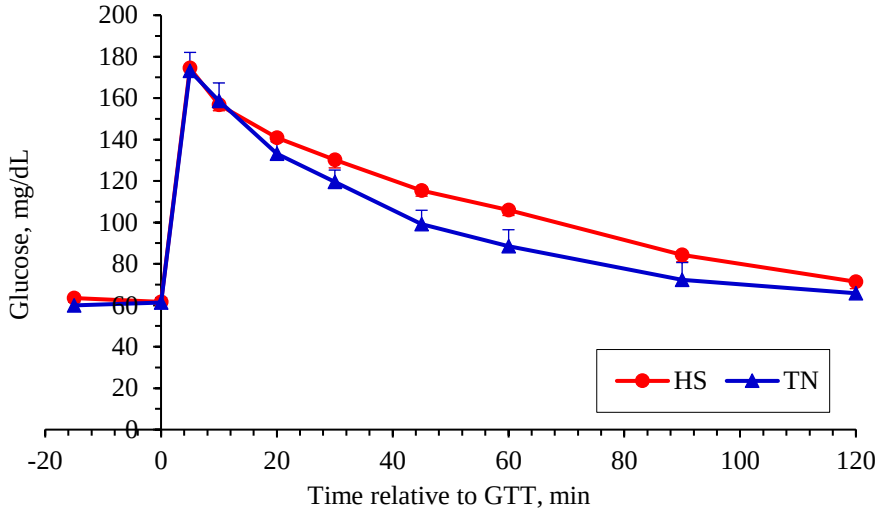


Figure 4.14. Glucose response to glucose tolerance test (GTT) of Manchega dairy ewes in late lactation under thermoneutral (TN, n = 10) or heat stress (HS, n = 10) conditions at the end of the experimental periods.

Table 4.9. Glucose and insulin responses to glucose tolerance test in Manchega dairy ewes under thermoneutral (TN, n = 10) or heat stress (HS, n = 10) conditions.

	Treatments			HS vs.TN	Effect (*P*-value)		
Item	TN	HS	±SEM	change, %	Treatment	Period	T×P[1]
Glucose							
Basal, mg/dL	60.7	62.8	1.7	3	0.38	0.28	0.84
Peak, mg/dL	173.1	174.5	6.6	1	0.89	0.62	0.52
Clearance[2], %/min	1.89	1.86	0.10	–2	0.83	0.34	0.68
AUC[3],							
min 60	7259	7750	254	7	0.19	0.34	0.32
min 120	11881	12972	545	9	0.17	0.15	0.24
Insulin							
Basal, µg/L	0.373	0.360	0.038	–3	0.82	0.95	0.08
Peak, µg/L	2.314	2.544	0.332	10	0.63	0.41	0.79
Clearance[4],%/min	1.82	1.38	0.21	–24	0.14	0.51	0.22
AUC[3],							
min 60	101	103	13	2	0.94	0.37	0.74
min 120	134	143	16	7	0.71	0.67	0.46
QUICKI[5]	0.376	0.379	0.009	1	0.82	0.84	0.60

[1]Treatment × period interaction, [2]From min 5 to 60; [3]Area under the curve; [4]From min 10 to 60; [5]Quantitative insulin-sensitivity check index.

Insulin Response to GTT. Values of insulin secreted in response to glucose administration are shown in Table 4.9 and Figure 4.15. Plasma insulin level increased in both groups similarly after the glucose injection and decreased gradually thereafter, reaching the basal levels at min 120. Insulin peaks were observed at min 10 in both groups, although the peak was numerically greater (+10%, $P = 0.63$) in HS than TN conditions. Moreover, the slope of the curve and the insulin clearance were slightly lower in the HS (–24%; $P = 0.14$; Table 4.9) than in the TN ewes.

Given that ewes under HS conditions had similar insulin AUC ($P = 0.71$ to 0.94; Table 4.9), but numerically greater glucose AUC ($P = 0.17$ to 0.19), this would suggest some degree of decreased insulin sensitivity in case of animals exposed to HS. This finding agrees with previous reports using insulin secretagogues in heat-stressed cows (Itoh et al., 1998), and is considered as a mechanism to have more glucose availability, which is apparently the favored fuel under HS conditions (Wheelock et al., 2010).

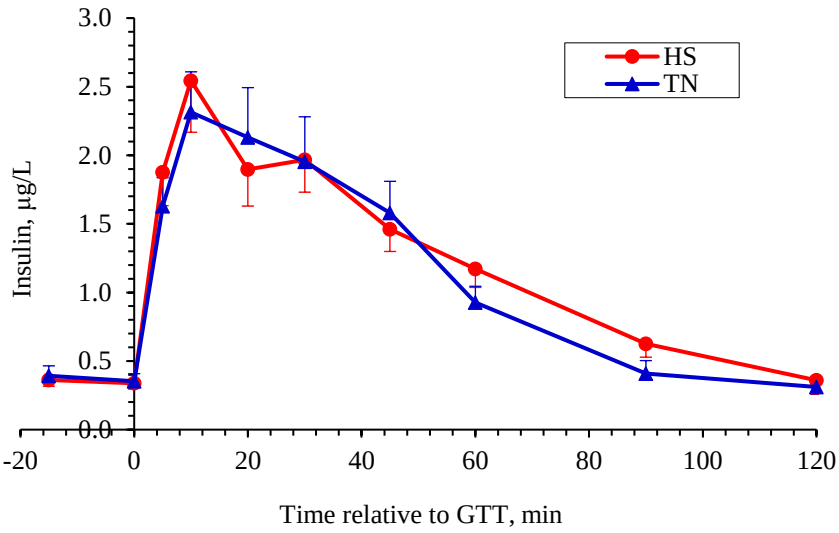


Figure 4.15. Insulin response to glucose tolerance test (GTT) of Manchega dairy ewes in late lactation under thermoneutral (TN, n = 10) or heat stress (HS, n = 10) conditions at the end of the experimental periods.

4.4.5.2. Tolerant vs. Sensitive Phenotypes

Glucose Response to GTT. Basal glucose values were similar for both phenotypes (Table 4.10). Plasma glucose level increased in both phenotypes after glucose injection and reached the highest level at min 5 (Figure 4.16a). However, the peak value tended to be lower ($P < 0.10$) in

the sensitive compared with the tolerant phenotype under TN conditions only. Additionally, glucose clearance rate tended to be lower (P = 0.061) in S ewes than T ewes, especially under TN conditions. The glucose area under curve was numerically (P = 0.17 to 0.23) lower in S ewes compared to T ewes under TN conditions (Figure 4.16a).

Table 4.10. Glucose and insulin responses to a glucose challenge of Manchega dairy ewes of different heat tolerance phenotypes (tolerant, T; sensitive, S) in late lactation under thermoneutral (TN) or heat stress (HS) conditions throughout the experiment.

	TN		HS				Effect (P-value)	
Item	T	S	T	S	±SEM	T[1]	Ph[2]	T×Ph[3]
Glucose								
Basal, mg/dL	58.9	62.4	61.5	64.0	2.3	0.38	0.25	0.96
Peak, mg/dL	187.9	158.3	174.2	174.7	8.2	0.87	0.10	0.09
Clearance[4], %/min	2.09	1.66	1.91	1.85	0.12	0.97	0.06	0.16
AUC[5],								
min 60	7684	6722	7736	7745	368	0.13	0.17	0.19
min 120	12457	11086	13170	12631	853	0.17	0.23	0.62
Insulin								
Basal, µg/L	0.373	0.372	0.354	0.366	0.058	0.83	0.96	0.91
Peak, µg/L	2.385	2.243	2.450	2.638	0.487	0.64	0.96	0.74
Clearance[6], %/min	1.758	1.890	1.234	1.618	0.335	0.21	0.40	0.69
AUC[5],								
min 60	107.4	93.7	109.8	95.8	19.1	0.91	0.46	0.99
min 120	141.8	122.7	160.8	124.4	23.2	0.65	0.23	0.72
QUICKI[7]	0.377	0.376	0.375	0.383	0.013	0.86	0.65	0.73

[1]Treatment, [2]Phenotype, [3]Treatment × phenotype interaction, [4]From min 5 to 60; [5]Area under the curve; [6]From min 10 to 60; [7]Quantitative insulin-sensitivity check index.

Insulin Response to GTT. Basal insulin values during the GTT did not vary (Table 4.10) and peaked similarly at 10 min in both phenotypes (Figure 4.17b) with no differences in clearance rates (Table 4.10). Insulin AUC at 60 and 120 min were 15% and 30% greater in T than in S ewes under HS conditions, but this difference did not approach significant level (P > 0.05). Having greater insulin AUC in T ewes under HS conditions with similar glucose availability in both groups (Figure 4.17a, b) could indicate greater insulin resistance under HS conditions in animals classified as heat tolerant.

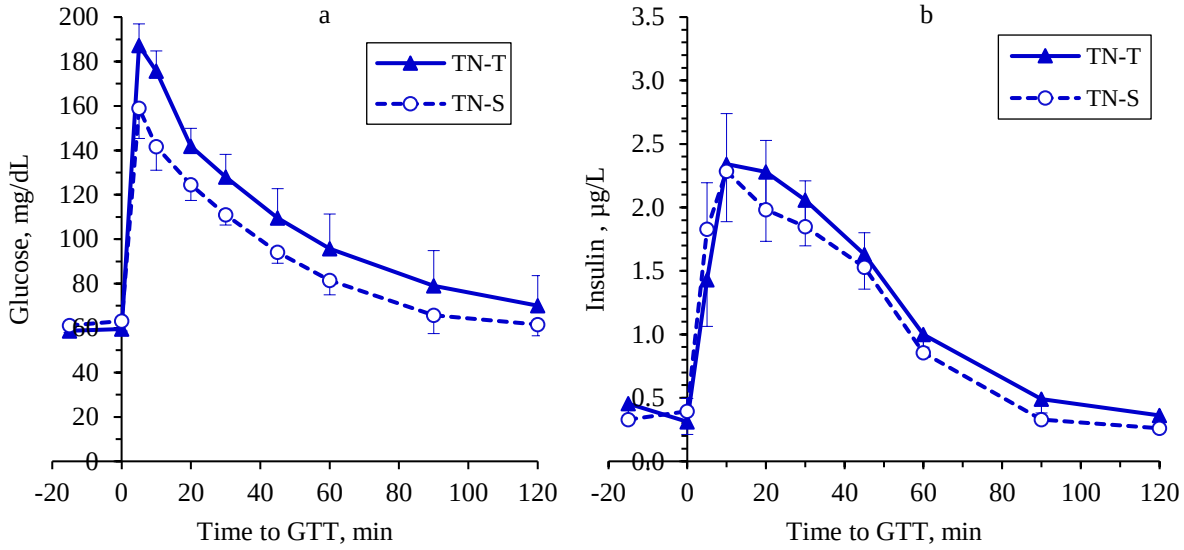


Figure 4.16. Glucose (a) and insulin (b) responses to glucose tolerance test (GTT) in Manchega dairy ewes of different heat tolerance phenotypes (tolerant, T; sensitive, S) in late lactation, under thermoneutral (TN) conditions.

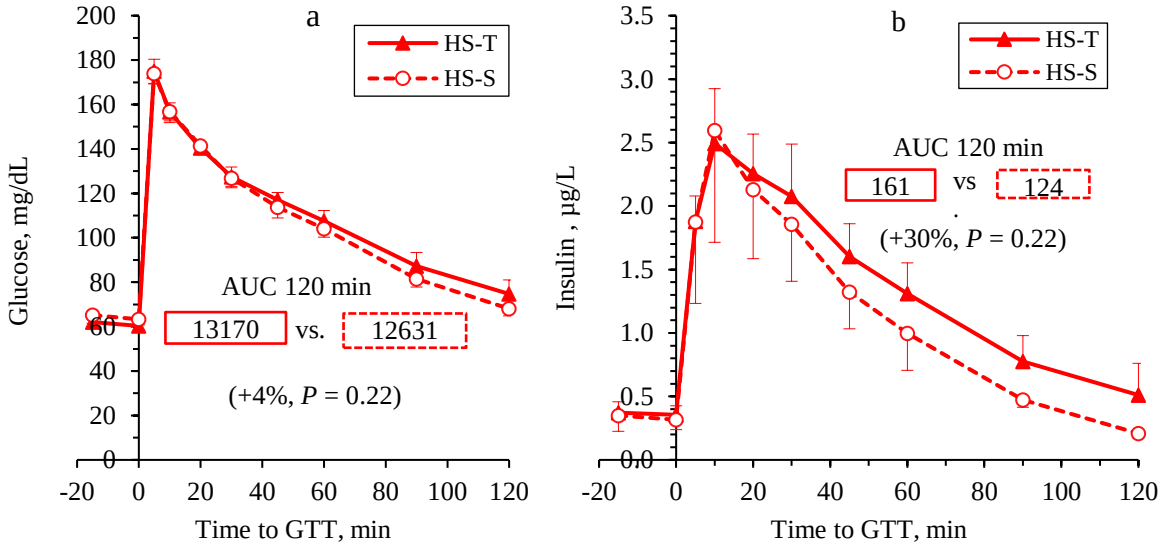


Figure 4.17. Glucose (a) and insulin (b) responses to a glucose tolerance test (GTT) in Manchega dairy ewes of different heat tolerance phenotypes (tolerant, T; sensitive, S) in late lactation under heat stress (HS) conditions.

4.5 CONCLUSIONS

Heat stress caused marked changes in thermophysiological traits in Manchega dairy ewes, including significant increments in rectal temperature and respiratory rate. Moreover, HS ewes increased water consumption and reduced feed intake. Nevertheless, milk yield and milk composition were not affected by HS. When comparing tolerant and sensitive phenotypes under HS conditions, the tolerant animals experienced less increments in rectal temperature and water consumption, which could indicate different mechanisms to cope with heat stress. One of these mechanisms could be greater insulin resistance in tolerant animal, and consequently more ability to keep normal blood glucose levels.

CHAPTER 5

Liver transcriptomics of sensitive and heat tolerant dairy sheep phenotypes

CHAPTER 5

Liver transcriptomics of sensitive and heat tolerant dairy sheep phenotypes

5.1 ABSTRACT

Detrimental effects of heat stress (HS) on animal welfare, productive and reproductive performances are well documented. The HS response includes not only physiologic, metabolic, and behavioral adjustments, but also a reprogramed transcriptome by cells. Liver has crucial role in metabolic responsiveness to cope with HS, but research on liver transcriptomic response is scarce in dairy sheep. Thus, the objective of the present study was to evaluate liver transcriptomics under HS in dairy ewes differing in heat tolerance phenotype. Multiparous Manchega dairy ewes phenotypically classified as tolerant (T; n = 5) or sensitive (S; n = 5) in late lactation (1.14 ± 0.07 kg/d, 154 ± 8 DIM) were used. The experimental design was a crossover of 2 periods (3 wk each) with 2 treatments: 1) thermo-neutral (TN; 15 to 20°C and 50 to 60% relative humidity; THI = 63 to 65), and 2) heat stress (HS; 12-h day at 37°C and 50% relative humidity; THI = 87; and 12-h nighty at 30°C and 50% relative humidity; THI = 79). Ewes were switched to the opposite treatment during the second period after a washout period of 2 wk. Liver tissue samples were obtained at the last day of each period for transcriptome analyses. The differential gene expression analysis was carried out with DESeq2 package of R environment. The effect of treatment (TN-T, TN-S, HS-T, HS-S), period (1, 2), and their interaction were included in the model. Gene Ontology (GO) and functional enrichment analyses were carried out in bioinformatic tool DAVID database. The comparison between T and S ewes under HS conditions revealed 1318 genes differentially expressed (893 downregulated and 425 upregulated). The downregulated genes were enriched in GO terms related to protein transport, extracellular exosome, cytosol, endoplasmic reticulum, and protein binding. Further, the downregulated pathways included endocytosis, various types of N-glycan biosynthesis, and complement and coagulation cascades. In conclusion, liver transcriptomic profile differed between T and S ewes when subjected to HS conditions, with the T ewes being less impacted by HS compared with the S ewes.

5.2 INTRODUCTION

Given the predicted rise in global temperatures, heat stress (HS) has become a major challenge affecting welfare, productive and reproductive performances of livestock (Baumgard and Rhoads, 2013; Lacetera, 2019), causing significant economic losses in dairy industry (St-Pierre et al., 2003). Strategies to alleviate the impact of HS include genetic development of heat tolerant breeds (Beed and Collier, 1986) and at the long-term, breeding of heat tolerant animals could be an effective remedy for reducing HS effects. To achieve this, it is necessary to identify those genes whose expression is changed in response to HS (Renaudeau et al, 2012). Applying this strategy considering the antagonism between milk yield and heat tolerance in dairy animals (West, 2003; Finocchiaro et al., 2005) would contribute to diminishing the detrimental effects of HS.

The response to HS includes physiological, metabolic, and behavioral adjustments, and involves cellular mechanisms that in turn are regulated by genes to restore homeostasis. These cellular mechanisms can be identified by the study of complete set of transcripts in cells using different high throughput technologies (Gracey 2007; Lowe et al., 2017,). In this sense, transcriptomics has emerged (specially using RNA-Seq technology) as a powerful approach for exploring the molecular response to stress factors such as HS (Cossins et al., 2006; Wang et al., 2009) and for identifying regulatory genes in heat tolerant animals.

The research based on transcriptome have yielded insights into the molecular basis of response to HS using different tissues such as blood (Lacetera et al., 2006; Do Amaral et al., 2010; Liu et al., 2020), mammary gland (Gao et al., 2019; Salama et al., 2019; Yue et al., 2020), liver (Shahzad et al., 2015), and muscle (Koch et al., 2016) from dairy ruminants exposed to HS compared with TN conditions. As reviewed by Collier et al. (2008), changes in gene expression patterns in response to acute HS include activation of heat shock transcription factor (HSF1), upregulation of heat shock proteins (HSP), and downregulation of protein synthesis. In addition to heat shock response, the deactivation of fatty acids and glycan synthesis, coupled with upregulated biosynthesis and degradation of amino acids (AA) were detected in liver transcriptomics from dairy cows during summer season (Shahzad et al., 2015).

Blood transcriptomics revealed that the dysfunction of immune cells, downregulation of lipid metabolism and tissue repair in Murciano-Granadina dairy goats were some of the HS responses (Contreras-Jodar et al., 2018). Further, the downregulation of candidate genes related to somatotropic axis, including growth hormone, insulin-like growth factor, and leptin in heat exposed Malabari goats were detected by liver transcriptomics (Angel et al., 2018). Regarding sheep, data available of Hu breed transcriptomics identified metabolic pathways, biosynthesis of AA, fat metabolism, and immunoreaction among other processes involved in HS response (Li et al., 2019; Lu et al., 2019). The multi-tissue transcriptome analysis (hypothalamus, liver, and ovary) of 2 sheep breeds differing in heat tolerance (Turpan black and Kazakh) have revealed differences in gene expression and pathways associated with energy metabolism and steroidogenesis under HS conditions (Haire et al., 2022).

The liver´s pivotal role in carbohydrate metabolism, lipid metabolism, and proteins secretion, such as albumin, transferrin, clotting factors, complements proteins and acute-phase proteins (Kubes and Jenne, 2018; Schulze et al., 2019) makes it a candidate dynamic tissue to study the impact of HS. Furthermore, the potential metabolic crosstalk of liver with different body organs, including muscle and adipose tissue, (Rui, 2014; Severinsen et al, 2020), makes it important to evaluate changes happening in liver in HS conditions. Evaluating these changes will help in understanding alterations in the biological process by HS and may help in establishing effective strategies to ameliorate performance of heat-stressed animals.

Aforementioned results refer mostly to dairy cows and little data are available on dairy ewes. Furthermore, few studies evaluated the response to HS according to the heat tolerance phenotype in the same breed. Given that the phenotype of an organism is essentially determined by the gene expression and gene complexes coordinated both in time and space (Gracey 2007), we hypothesized that response of dairy ewes to HS in terms of gene expression would differ according to their heat tolerance phenotype. Consequently, the objective of the present study was to evaluate liver transcriptome profile of Manchega dairy ewes differing in heat tolerance phenotype (tolerant vs. sensitive) under HS conditions.

5.3 MATERIALS AND METHODS

The experimental procedures were approved by the Ethics Committee of Animal and Human Experimentation of the Universitat Autonoma de Barcelona, UAB (Ref. 3142) and agreed with the codes of recommendations for the welfare of livestock of the Ministry of Agriculture, Food and Environment of Spain.

5.3.1. Animals, Management Conditions, and Treatments

Ten multiparous Manchega dairy ewes in late lactation (1.14 ± 0.07 kg/d milk yield, 154 ± 8 DIM, and 67.5 ± 1.2 kg BW) from the herd of the Servei de Granges i Camps Experimentals (SGCE) of the UAB were individually penned in climatic chamber (see Chapter 4.3). Five ewes were phenotypically classified as tolerant (T) and five ewes were sensitive (S) to HS according to heat tolerance test described in Chapter 4.3.

The experimental design was a crossover of 2 periods (3 wk each) with 2 treatments: 1) thermoneutral (TN; 15 to 20°C and 50 to 60% relative humidity; THI = 63 to 65), and 2) heat stress (HS; 12-h day at 37°C and 50% relative humidity; THI = 87; and 12-h night at 30°C and 50% relative humidity; THI = 79). Photoperiod was maintained constant at 12-h light:12-h dark (0900 to 2100 h). Ewes were switched to the opposite treatment during the second period after a washout period of 2 wk. Liver tissue samples were obtained at the last day of each period for transcriptome analyses.

5.3.2. Sample Collection, Measurements, and Analyses

Liver Biopsies

Before the surgical procedure, the right flank was shaved from the 7^{th} to 12^{th} intercostal spaces with an electric shaver (Oster Golden model A5, Miami, FL). Each ewe was sedated by the i.v. injection of 0.15 mg/kg BW of xylazine hydrochloride (20mg/mL, Xilasol, Karizoo Laboratories, Barcelona, Spain).) Then, ewes were positioned in left lateral recumbency and 3 mL of Lidocaine 2% (20 mg/ml, B Braun, Barcelona, Spain) were s.c. administered at the site of biopsy. Visualization of the liver and insertion path of the biopsy needle were performed by ultrasound-guided technique using a Real time B-mode ultrasonography with a convex C60/5-2 MHz transducer (SonoSite Ultrasound System, Vet180 Plus, Bothell, WS). Biopsies were obtained using a semi-automatic VI Trucut type SuperCore TM 14G × 9 cm biopsy instrument

(Argon Medical Devices, Athens, TX). The liver specimens were snap frozen in liquid N and stored at −80°C until RNA extraction.

RNA Extraction and Quality Assessment

Total RNA of liver tissue samples was extracted using TRIzol® Reagent with the PureLink™ RNA Micro Kit (Invitrogen, Carlsbad, CA) according to the manufacturer´s instructions. The concentration and purity of RNA were determined by a NanoDrop™ 2000 spectrophotometer (ThermoFisher Scientific, Wilmington, DE). The RNA concentration averaged 246 ± 52 ng/µL and the mean of absorbance ratio 260/280 was 2.00 ± 0.01. RNA integrity number (RIN) was assessed using Fragment Analyzer System with DNF-471 RNA Kit (Agilent Technologies, Santa Clara, CA). The RIN values averaged 7.64 ± 0.33.

Library Construction and Sequencing

RNA-seq library preparation was performed according to Illumina protocol (TruSeq Stranded mRNA, Illumina, San Diego, CA). Poly-A tails were isolated from total RNA by Oligo-dT beads, and then mRNA was fragmented and primed for first and second strand cDNA synthesis. Adenylation was carried out in 3´s ends and adapters ligated to cDNA. Then DNA fragments were enriched and amplified by PCR. The libraires were run on HiSeq 4000 instrument (Illumina, San Diego, CA) for cluster generation and paired-end sequencing (2 × 75 bp), which generated 26.3 million of raw reads per library, on average.

Data Preprocessing and Alignment

Raw reads obtained after Illumina sequencing were subjected to quality control by the FastQC software v 0.11.9 (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/). Bases with Phred score < 25Q, reads with lower than 30 bp, and adapters were removed using TrimGalore 0.6.4 tool (https://www.bioinformatics.babraham.ac.uk/projects/trim_galore/). Additionally, 15 bp from both ends of each read were excised since sequencing errors occur frequently in these regions (Conesa et al., 2016). Thereafter clean reads (24.9 million reads per sample on average) were aligned to the Ovine reference genome (Oar_rambouillet_v1.0; https://www.ncbi.nlm.nih.gov/assembly/GCF_002742125.1/) using Hisat2 (version 2.1.0). A guided transcripts assembly and subsequent quantification were carried out with StringTie

(version 2.1.1), whereas matrix building containing the read counts per transcript and sample was performed with Python3 (version 3.8.2). Table 5.1 summarizes sequenced RNA-Seq data.

Table 5.1. Summary statistics of sequence and alignment information of liver samples from TN and HS treatments.

	Treatment	
Item	TN	HS
Raw reads, n	25,115,926	27,577,284
Clean reads, n	23,781,276	26,068,631
Clean reads, %	93.7	93.7
GC content, %	48.0	46.0
Mapped reads, n	21,267,931	23,192,986
Mapped reads, %	93.6	93.4
Uniquely mapped reads, n	16,246,507	17,274,184
Multi mapped reads, n	5,021,424	5,918,802
Unmapped reads, n	2,513,345	2,875,645

5.3.3. Differential Gene Expression Analysis

In order to identify differentially expressed genes (DEG) between TN and HS conditions, and T and S phenotypes, the DESeq2 package (version 1.30.0) from Bioconductor software project (https://www.bioconductor.org/packages/release/bioc/html/DESeq2.html) in R (version 4.0.3) was used. The effect of treatment (TN-S, TN-T, HS-S, and HS-T), period (1 and 2), and their interaction were included in the model. To evaluate the effects of HS regardless the phenotype, the treatment variable contained only 2 levels (TN vs HS). Log_2 fold change (log_2FC) > 1.5 was set to detect the DEG. The false discovery rate (FDR) was controlled according to Benjamini-Hochberg test (Benjamini and Hochberg, 1995) and was set at *q*-value < 0.05.

5.3.4. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) Pathways Analyses

After gene IDs conversion, the lists of DEG were uploaded into the Database for Annotation, Visualization, and Integrated Discovery (DAVID) version 2021 (https://david.ncifcrf.gov/) for the GO and functional enrichment analysis. With this aim, the enrichment *q*-value ≤ 0.05 and minimum gene counts of 3 were the thresholds applied.

5.4 RESULTS AND DISCUSSION

5.4.1. Identification of Differentially Expressed Genes in Response to HS

The analysis revealed 40 DEG of which 1 was upregulated (ITGAD) and 39 were downregulated (Table 5.2) in HS compared to TN conditions. This low gene expression level could be attributed to the fact that liver biopsies were collected at the last day of each period (d 21), where acclimation or metabolic adjustments had been taken place to minimize the detrimental effects of HS (Collier et al., 2008). Thus, our results exhibit chronic gene expression rather than acute response. With this regard, it was reported that acute stress induced stronger transcriptomic response in terms of DEG number than chronic stress in liver of chickens and hens (Lan et al., 2016, Wang et al., 2021).

Table 5.2. Top 20 downregulated genes of liver tissue in Manchega dairy ewes heat-stressed for 21 d compared with thermal neutral counterparts.

Gene symbol	Entrez_gene_ID	*q*-value	LFC[1]
MYLPF	100270715	1.81E-03	-30.00
RYR1	101112591	2.79E-04	-28.79
MB	780509	2.50E-03	-28.69
XIRP2	101108995	3.35E-03	-28.19
TNNC1	101111132	4.22E-03	-27.79
MYL2	100196904	6.65E-03	-27.12
SLN	101104853	8.23E-03	-26.62
TNNI1	101112151	8.23E-03	-26.49
MYOM2	101102872	1.09E-02	-25.78
TRIM54	101111790	1.13E-02	-25.65
LMOD2	101106254	1.13E-02	-25.54
TXLNB	101115628	1.18E-02	-25.32
AMPD1	101102023	1.44E-02	-25.01
PPP1R3A	101110321	1.89E-02	-24.41
ARPP21	101106887	2.21E-02	-24.18
TRIM72	101106488	2.21E-02	-24.14
KLHL40	101119589	2.93E-02	-23.66
TBX15	101110282	2.93E-02	-23.61
TRIM63	101113132	2.94E-02	-23.59
VGLL2	101103729	3.83E-02	-23.17

[1]Log_2 fold change

The upregulated gene ITGAD (Integrin subunit alpha D) by HS belongs to a large integrins family of membrane glycoproteins and it encodes the alpha subunit of the cell surface heterodimers (NCBI, 2022). Thus, ITGAD is involved in several biological processes related to cell adhesion, integrin mediated signaling pathway, extracellular matrix organization, and regulation of actin cytoskeleton pathway. Disruptions of cell organization including actin cytoskeleton rearrangement in response to heat shock have been reported in a variety of cell cultures as rat fibroblasts (Welch and Suhan, 1985), mouse mammary epithelial cells (Shyy et al., 1989) and bovine embryos (Rivera et al., 2004), and the accumulating defects are dependent on duration and intensity of HS (Richter et al., 2010). The fact that ITGAD gene was upregulated in our HS ewes could be considered as an adaptive response of hepatocytes, where the rearrangement of cytoskeleton was in progress.

The MYLPF, RYR1, MB, MYL2, SLN and TNNC1 downregulated genes by HS in our ewes (Table 5.2) were upregulated by cold stress in liver transcriptome of Altay lambs (Jiao, et al., 2021). Those authors suggested that liver is capable to regulate muscular shivering (ST) and no shivering (NST) thermogenesis and enhanced liver mediated muscle metabolism by muscle contraction and transition between fast and slow fibers in response to cold stress.

Pant et al. (2016) described how sarcolipin (SLN) is essential for muscle (skeletal and cardiac) thermogenesis, body temperature maintenance and metabolism via regulation of SERCA pump (sarcoplasmic/endoplasmic reticulum Ca^{2+} ATPase). Interestingly, the gene that encodes sarcolipin (SLN) is in the top 10 downregulated genes (Table 5.2) in the liver tissue from our HS ewes and it was also detected in the top 50 upregulated genes (liver tissue) in lambs under cold stress (Jiao, et al., 2021). Under cold conditions, higher level of SLN uncouples SERCA pump, increase ATP hydrolysis leading to heat production (Smith et al., 2002), which is contrary to what should happen in HS conditions.

Skeletal muscle is one of the most abundant tissues in mammals and has a major metabolic function and given its ability to generate heat through ST and NST mechanisms, muscle can play important homeostatic roles in thermogenesis as well as in whole body metabolism by regulating fuel utilization (Pant et al., 2016). In this sense, our heat-stressed ewes decreased DMI and increased muscular breakdown (higher level of plasma creatinine, Chapter 4) to provide gluconeogenic substrates as AA for the liver. The metabolic crosstalk between liver and

other tissues as muscle and adipose tissue has been evaluated (Rui, 2014; Severinsen et al, 2020), but at the gene transcriptional level, limited information is available for liver-muscle crosstalk during stressful conditions such as HS. There is evidence that liver responds to acute exercise, resulting in marked transcriptional upregulation of stress response genes in rats (Hoene and Weigert, 2010). Further, liver transcriptomics from heat-stressed chickens revealed enriched GO terms related to contractile fiber part, sarcomere, and myofibril among others (Lan, et al., 2016).

The presented results herein show the transcriptional crosstalk between liver and muscle, where liver seems to act as a regulator of muscle tissue, especially muscular NST response. In case of HS ewes, the NST response was downregulated, most probably to avoid extra metabolic heat production. Furthermore, the genes involved in thermoregulation (MYLPF, RYR1, ACTN3, MYOM2, TNNC1, and SLN) were downregulated.

Table 5.3. Functional annotations using the 39 genes that differentially expressed between dairy ewes exposed to thermoneutral or heat stress conditions for 21 d.

Item	Gene count	Change	Fold enrichment	*P*-value	*q*-value
Biological process					
Muscle contraction	7	Down	61.7	9.30E-10	2.00E-07
Transition between fast and slow fiber	3	Down	255.4	5.40E-05	5.70E-03
Ventricular cardiac muscle tissue morphogenesis	3	Down	92.0	4.40E-04	3.10E-02
Embryonic cranial skeleton morphogenesis	3	Down	74.2	6.80E-04	3.60E-02
Sarcomere organization	3	Down	58.9	1.10E-03	4.60E-02
Cardiac muscle contraction	3	Down	52.2	1.40E-03	4.90E-02
Cellular component					
Z disc	5	Down	27.3	2.70E-05	1.90E-03
M band	3	Down			
Molecular function					
Structural constituent of muscle	4	Down	62.3	3.20E-05	2.10E-03

The enriched GO terms in response to HS conditions were related to muscle contraction, transition between fast and slow fiber, ventricular cardiac muscle tissue morphogenesis, sarcomere organization, structural constituent of muscle, among others (Table 5.3). Figure 5.1 represents gene network of the 3 main downregulated biological processes (BP): muscle contraction, transition between fast and slow fiber, and ventricular cardiac muscle tissue

morphogenesis. These findings support the idea of liver-muscle cross-talking under HS conditions.

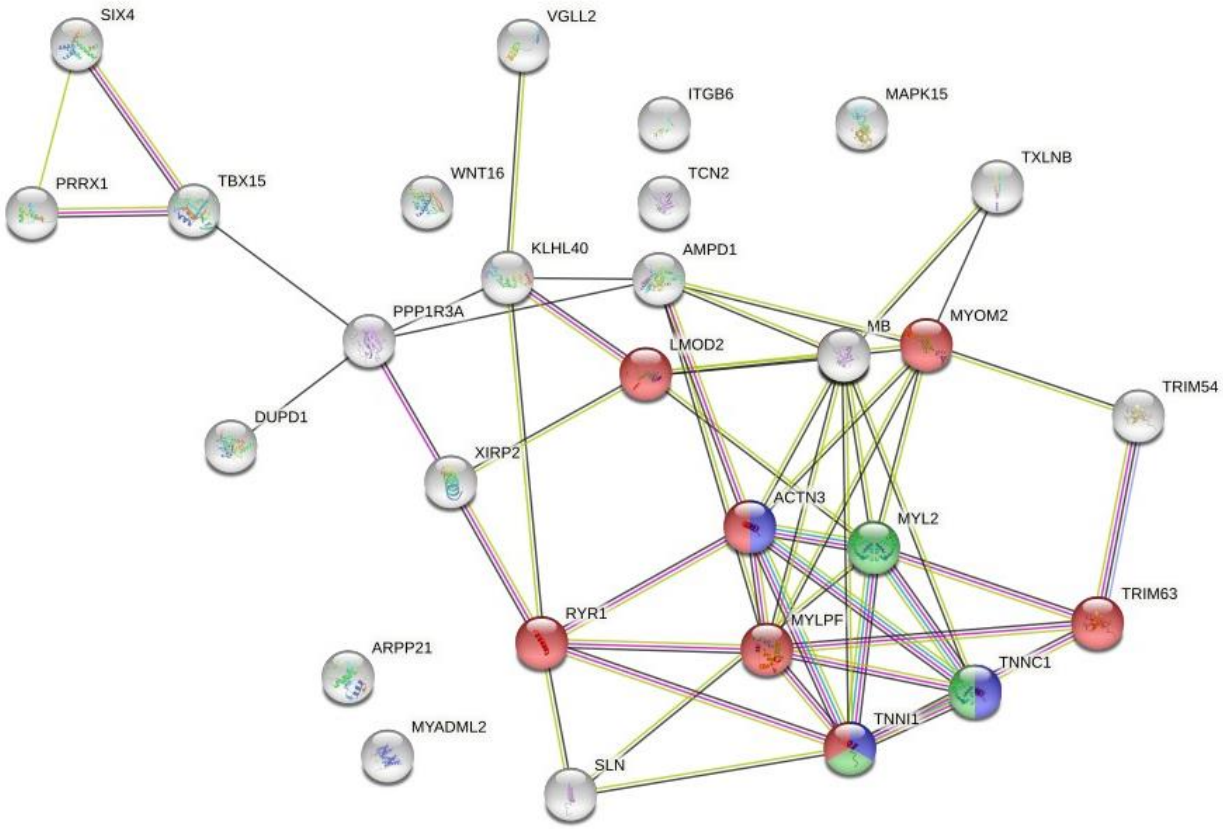


Figure 5.1. Gene interaction network of downregulated genes under HS. Nodes represent genes related to biological processes (BP) of muscle contraction (red), transition between fast and slow fiber (blue) and ventricular cardiac muscle tissue morphogenesis (green). Edges are the interactions between nodes.

5.4.2. Identification of Differentially Expressed Genes (DEG) in Tolerant vs. Sensitive Ewes under HS Conditions

We have sequenced 20 RNA samples corresponding to T (n = 10) and S (n = 10) phenotypes with 5 samples of each phenotype in each period. A total of 22,226 genes were found to be expressed in at least one of the 20 samples analyzed. By establishing q-value < 0.05 and $\log_2$FC > 1.5, we detected 1318 DEG between T and S phenotypes under HS conditions (Figure 5.2). Furthermore, Figure 5.3 represents the heatmap of the DEG in all treatment groups and periods. According to the heatmap, period had strong effect on gene expression profile since 6 wk were elapsed between biopsies in the 1st and 2nd experimental periods. This led to changes

in the stage of lactation and the metabolic status, which affected gene expression in the liver. However, to identify the DEG, the effects of period and its interaction with phenotype were included in the statistical model (see materials and methods for details). Out of the 1318 DEG, 425 (32%) were upregulated and 893 (68%) were downregulated with LFC from −21.8 to 29.6. Tables 5.4 and 5.5 list the top 20 down and upregulated genes, respectively.

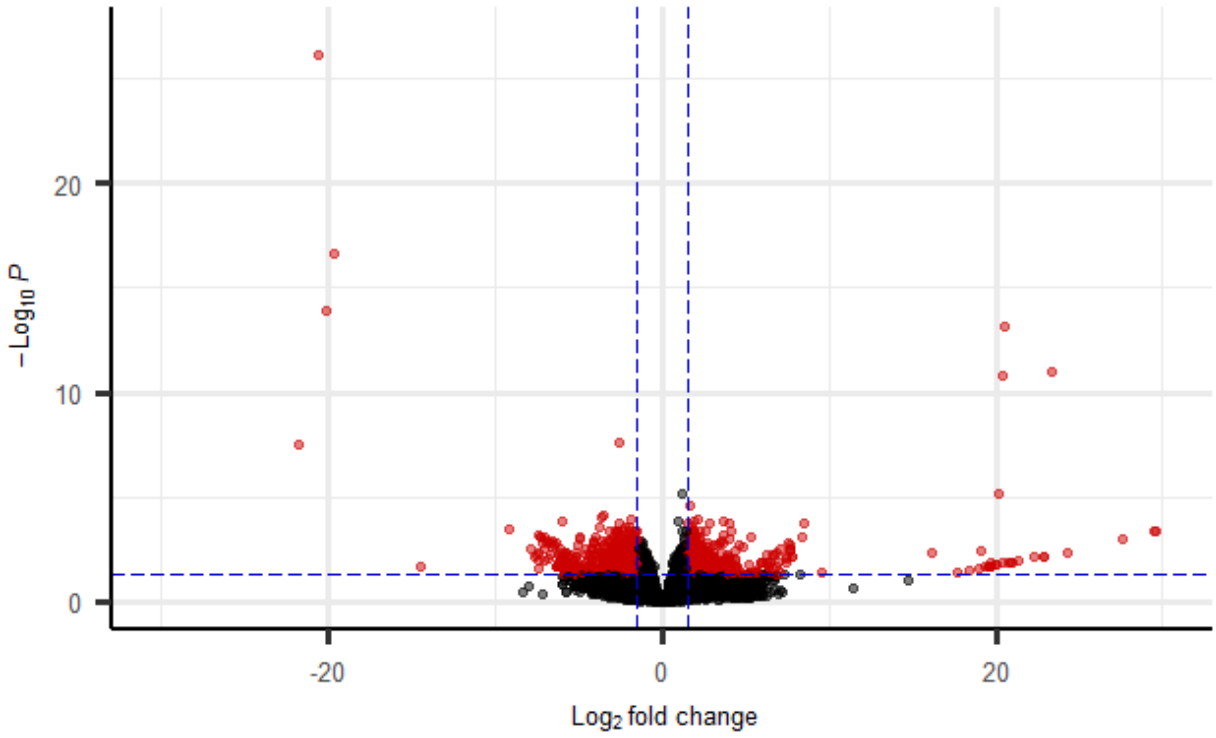


Figure 5.2. Volcano-plot showing genes that were differentially expressed with adjusted *P*-value < 0.05 in tolerant and sensitive ewes under HS conditions.

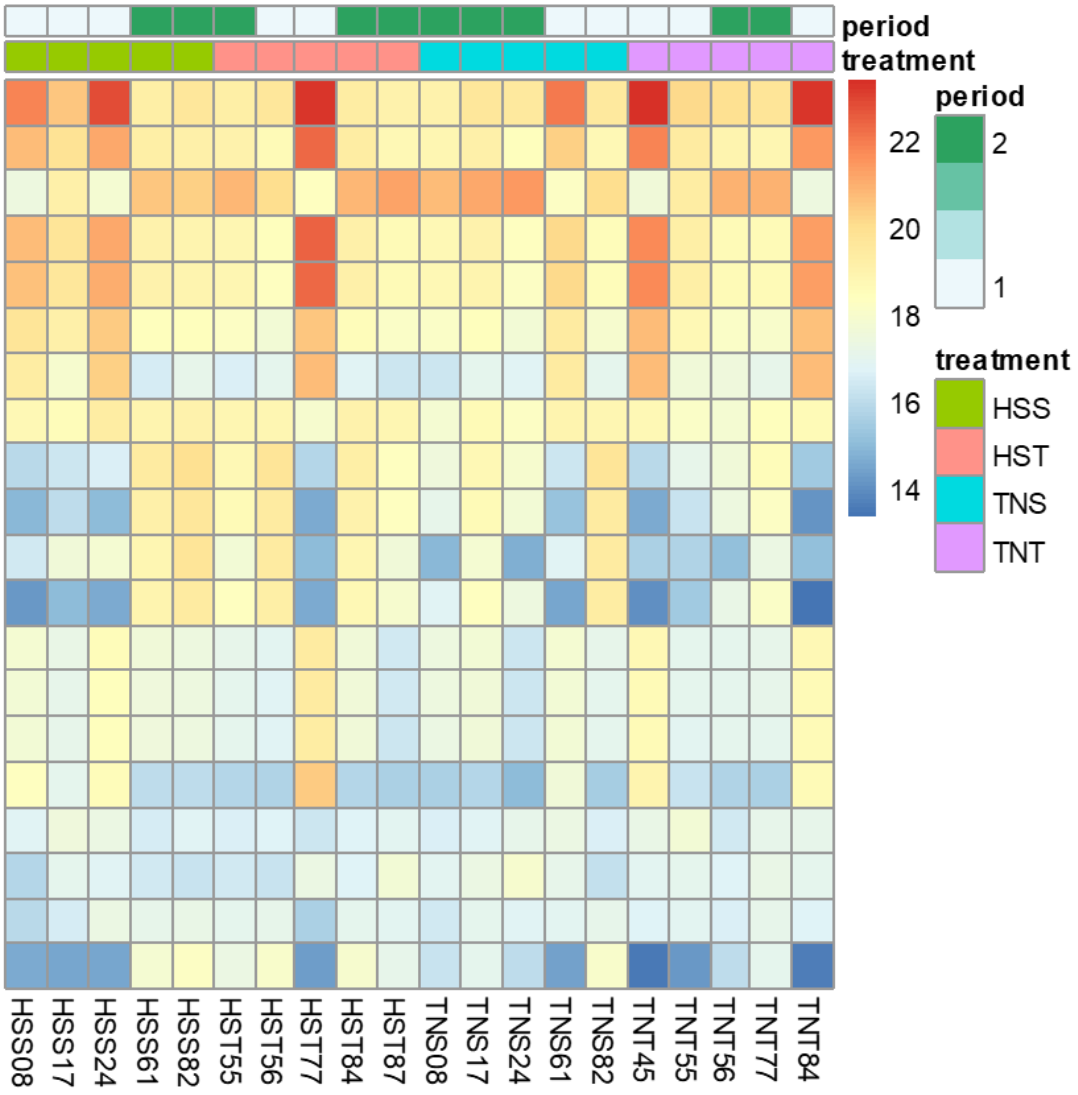


Figure 5.3. Heat map of gene expression profile in tolerant (T) and sensitive (S) ewes under thermoneutral (TN) and heat stress (HS) during the first (1) and second (2) experimental periods.

Phosphoprotein 1 (SPP1) gene was the most downregulated gene in heat-stressed T animals compared to the S phenotype (Table 5.4). This gene encodes the osteopontin (OPN) protein, which has well-established several physiological roles and is also involved in chronic liver disease. Specifically, OPN binds lipopolysaccharide (LPS), lowers oxidative stress, and maintains the homeostasis of intestinal commensal bacteria (Song et al., 2021). Heat stress is known to increase cell oxidative stress (Chauhan et al. 2014) and to induce chronic systemic

proinflammatory response because of the leaky intestine and LPS release to the circulation (Contreras-Jodar, 2019). Thus, it seems that T animals did not suffer such conditions and did not need OPN.

Table 5.4. Top 20 downregulated genes of liver tissue in tolerant vs. sensitive Manchega dairy ewes under heat stress conditions.

Gene symbol	Entrez_gene_ID	*q*-value	LFC[1]
SPP1	443058	2.8E-08	-21.76
LEPR	443264	7.5E-27	-20.62
RPP14	101122389	1.2E-14	-20.11
LOC105616215	105616215	2.2E-02	-14.42
LOC101108817	101108817	3.2E-04	-9.13
LOC101117971	101117971	2.6E-03	-7.83
LOC101108321	101108321	6.3E-03	-7.65
RASA2	101108362	6.0E-03	-7.46
EDIL3	101118698	1.1E-02	-7.38
MANEA	101106778	5.9E-04	-7.36
LYPD5	101108855	7.8E-04	-7.34
SLC30A7	100302550	7.6E-04	-7.34
MIPOL1	101121886	1.4E-03	-7.18
LEKR1	101120544	9.9E-03	-6.99
CTSL	101113725	2.8E-03	-6.99
ROR1	101109659	1.3E-03	-6.91
TFEC	101109437	1.5E-03	-6.81
ARHGAP19	101113027	5.4E-03	-6.72
IL7	443341	1.4E-03	-6.70
SAC3D1	101105453	1.2E-03	-6.54

[1]Log_2 fold change

Leptin-receptor (LEPR) gene was downregulated in tolerant ewes compared to the sensitive phenotype (Table 5.4). The activation of LEPR by leptin contributes to hepatic inflammation and fibrosis through the increase of growth factors and proinflammatory and proangiogenic cytokines (Polyzos et al., 2015). Leptin may protect from the accumulation of lipids in the liver; however, if the disease progresses, leptin may worsen it by acting as an inflammatory and fibrogenic factor (Polyzos et al., 2015). This could be explained by the induction of fatty acid oxidation by leptin and the mitochondrial respiration resulting in a state of oxidative stress. Although not significant, S ewes experienced greater numerical levels ($P < 0.28$; +27%) of blood NEFA compared to T ewes in HS (Table 4.7, Chapter 4).

Table 5.5. Top 20 upregulated genes of liver tissue in tolerant vs. sensitive Manchega dairy ewes under heat stress conditions.

Gene symbol	Entrez_gene_ID	*q*-value	LFC[1]
PEBP4	101113986	4.10E-04	29.61
ABRA	101123385	4.13E-04	29.52
UCP3	443278	1.07E-03	27.59
GRID1	101114332	7.22E-03	22.87
IP6K3	101118907	7.69E-03	22.31
RNF207	101117952	1.07E-02	21.39
RXRG	101111845	1.27E-02	20.84
FHOD3	101115334	1.33E-02	20.72
TMOD1	101104717	7.03E-14	20.50
CACNG1	101118891	1.46E-02	20.44
PTP4A3	101120416	1.64E-11	20.44
ANO5	101120120	1.76E-02	19.97
GRIP2	101109395	1.46E-02	19.75
CACNG6	101118891	1.90E-02	19.72
CAV3	101107228	1.90E-02	19.72
SGCG	101116584	2.01E-02	19.56
HOXD8	101103879	2.01E-02	19.55
SCN4B	101113622	2.15E-02	19.33
ART3	101108229	3.80E-03	19.16
NMRK2	101114607	2.37E-02	19.04

[1]Log_2 fold change

On the other hand, uncoupling protein-3 (UCP3) was upregulated (LFC = 28) in T compared with S ewes (Table 5.5). The UCP3 is not usually expressed in the liver and is only upregulated when fatty acid catabolism is increased (Silvestri et al., 2006). As indicated in Table 4.6 (Chapter 4), HS caused an increment (+74%, P = 0.015) in blood NEFA compared to TN conditions. Expression of UCP3 in the liver could be considered as a physiological reaction to protect hepatic cells against metabolic damage resulting from forced lipid metabolism with its related oxidative stress (Camara et al., 2009; Marra et al., 2008). It is most likely that T ewes protected their liver under HS conditions by the upregulation of UCP3 gene.

As shown in Table 5.5, inositol hexakisphosphate kinase (IP6K3) gene was upregulated (LFC = 22) in T compared to S phenotype. Mukherjee et al. (2020) reported that IP6K1 promotes insulin secretion from pancreatic β-cells and increases insulin resistance. This insulin resistance is induced because IP6K3 reduces insulin signaling in metabolic tissues by inhibiting the protein

kinase Akt. As discussed in Chapter 4, T ewes secreted more insulin in response to GTT (Figure 4.17a, Chapter 4) and seem to have greater insulin resistance than S ewes in HS conditions.

5.4.3. Gene Ontology (GO) and Enriched Pathways Analyses in Tolerant and Sensitive Ewes under HS Conditions.

The GO terms classified as biological process, cellular component, and molecular function, along with the enriched pathways are presented in Table 5.6. The GO terms were mainly related to protein transport, extracellular exosome, cytosol, endoplasmic reticulum (ER), and protein binding. These results indicate less protein turnover in T compared to S ewes, which could suggest a less impact of HS on A ewes. It is well documented that HS cause protein misfolding and aggregation resulting in an influx of misfolded or aggregated proteins to ER lumen (Bouchama et al., 2017).

The KEGG pathways of endocytosis, various types of N-glycan biosynthesis, and complement and coagulation cascades were downregulated in T compared with S ewes (Table 5.6). Plasma membrane shapes the interactions between cells and their environment through the transmembrane proteins (receptors, transporters, channels, or adhesion proteins). The dynamic remodeling of the cell surface proteome is likely a crucial process in the cellular adaptation to many stressful situations, including heat shock (López-Hernández et al., 2020). When cells are stressed, the fastest and most effective way to modify the cell surface protein profile is the alteration in endocytosis (Bitsikas et al., 2014). It should be kept in mind that stress conditions can either promote or hamper endocytosis. In our T tolerant ewes the endocytosis pathway was downregulated. Downregulation of endocytosis extends the residence time of signaling receptors and transporters at the cell surface, and thereby promoting their activities (López-Hernández et al., 2020) as the nutrient uptake by glucose transporters 1 and 4, and AA carriers (Antonescu et al., 2014).

Consequently, T ewes would have been more able to uptake nutrients (e.g., glucose and AA) and efficiently face the stress compared to S animals.

Table 5.6. Functional annotations and enriched KEGG pathways using the 1318 genes that differentially expressed between tolerant (T) and sensitive (S) ewes under heat stress conditions.

Item	Gene count	Change	Fold enrichment	*P*-value	*q*-value
Biological process					
Protein transport	35	Down	2.3	1.40E-05	4.60E-02
Cellular component					
Extracellular exosome	168	Down	2.2	1.20E-22	7.30E-20
Cytosol	277	Down	1.5	2.60E-13	8.30E-11
Membrane	143	Down	1.6	4.10E-09	8.50E-07
Endoplasmic reticulum	78	Down	2.0	8.10E-09	1.30E-06
Endoplasmic reticulum membrane	73	Down	2.0	1.00E-08	1.30E-06
Focal adhesion	36	Down	2.4	2.50E-06	2.20E-04
Perinuclear region of cytoplasm	53	Down	2.0	2.80E-06	2.20E-04
Cell surface	47	Down	2.1	2.80E-06	2.20E-04
Golgi membrane	46	Down	2.1	4.50E-06	3.20E-04
Cytoplasm	242	Down	1.3	5.40E-06	3.40E-04
Lysosome	28	Down	2.6	8.40E-06	4.80E-04
Membrane raft	25	Down	2.8	9.10E-06	4.80E-04
Platelet alpha granule	7	Down	11.7	1.60E-05	7.80E-04
Receptor complex	22	Down	2.9	2.60E-05	1.20E-03
Golgi apparatus	65	Down	1.7	3.40E-05	1.40E-03
Melanosome	14	Down	3.9	6.40E-05	2.50E-03
Nucleoplasm	177	Down	1.3	6.60E-05	2.50E-03
Azurophil granule lumen	13	Down	4.1	7.90E-05	2.80E-03
Trans-Golgi network	19	Down	2.9	1.20E-04	3.80E-03
Ubiquitin ligase complex	14	Down	3.6	1.40E-04	4.40E-03
Endosome	25	Down	2.3	2.80E-04	8.30E-03
Endosome membrane	21	Down	2.4	4.20E-04	1.20E-02
Lysosomal membrane	27	Down	2.1	4.90E-04	1.30E-02
Platelet alpha granule lumen	10	Down	4.3	5.30E-04	1.30E-02
Endoplasmic reticulum lumen	24	Down	2.2	5.50E-04	1.30E-02
Late endosome	15	Down	3.0	5.50E-04	1.30E-02
Azurophil granule membrane	9	Down	4.4	8.90E-04	2.00E-02
Intracell membrane-bounded organelle	51	Down	1.6	9.00E-04	2.00E-02
Cytoplasmic vesicle	24	Down	2.1	1.00E-03	2.20E-02
Endoplasmic reticulum exit site	7	Down	5.9	1.10E-03	2.20E-02
Cell-cell junction	17	Down	2.5	1.20E-03	2.40E-02
Filopodium	10	Down	3.6	1.80E-03	3.50E-02
Lysosomal lumen	11	Down	3.3	2.00E-03	3.80E-02
HFE-transferrin receptor complex	4	Down	14.2	2.10E-03	3.90E-02
Late endosome membrane	13	Down	2.8	2.50E-03	4.50E-02
Mitochondrial matrix	26	Down	1.9	2.70E-03	4.70E-02
Glutamatergic synapse	25	Down	1.9	2.80E-03	4.70E-02
Secretory granule membrane	11	Down	3.1	2.90E-03	4.80E-02

Table 5.6. (Continued)

Item	Gene count	Change	Fold enrichment	*P*-value	*q*-value
Molecular function					
Protein binding	553	Down	1.2	1.40E-13	1.30E-10
Signaling adaptor activity	8	Down	6.9	1.20E-04	5.30E-02
Cell adhesion molecule binding	11	Down	4.3	2.20E-04	5.30E-02
Growth factor binding	8	Down	6.1	2.60E-04	5.30E-02
Protein homodimerization activity	46	Down	1.8	3.10E-04	5.30E-02
Cadherin binding	26	Down	2.2	3.40E-04	5.30E-02
Enriched KEGG Pathways					
Endocytosis	25	Down	2.3	1.80E-04	2.60E-02
Various types of N-glycan biosynthesis	9	Down	5.4	2.00E-04	2.60E-02
Complement and coagulation cascades	13	Down	3.6	2.50E-04	2.60E-02

The ER is the primary site of protein synthesis, folding, maturation, quality control and degradation, and ensure only properly folded proteins are delivered (Almanza et al., 2019). The perturbation of ER homeostasis is called ER stress and occurs when there is accumulation of unfolded/misfolded protein or when ER Ca is depleted (Liu and Green 2019). Heat stress causes protein misfolding and aggregation, resulting in ER stress. To fix this, ER responds by activating intracellular signal transduction pathways collectively known as the unfolded protein response (UPR) (Walter and Ron, 2011). The main role of UPR is to restore ER homeostasis by 1) eliminating misfolded proteins and 2) reducing newly proteins synthesis in the ER (Jiang et al., 2021) with the goal of protecting cells against potential harmful effects of defective proteins (Almanza et al. 2019). Data from broiler models demonstrated UPR activation in liver transcriptome under acute heat stress (Miao et al, 2022). In this sense, protein glycosylation (e.g., N-glycan biosynthesis) is a posttranslational modification and considered as a part of the UPR to reduce ER stress. Protein glycosylation helps regulate protein homeostasis by changing the function of glycosylation to modify unfolded protein binding and protein processing in ER-related proteins (Gao et al., 2021). The ER stress also activates the complement and coagulation cascade pathway in hepatic cells (Wang et al., 2022). Additionally, the activation of the complement and coagulation cascade pathway has been shown to be positively correlated with the proinflammatory factors (Wang et al., 2022). In the current study, the downregulation of N-glycan biosynthesis, and the complement and coagulation cascade pathways in the T ewes may indicate that these ewes did not suffer major ER stress. Consequently, the T ewes needed fewer

corrective measures (e.g., protein glycosylation, and complement and coagulation cascades activation) compared with the S ewes.

5.5 CONCLUSIONS

Chronic HS (i.e., 21 days) caused few changes in liver transcriptome, with almost all the affected genes being downregulated. These changes in gene expression indicated crosstalk between hepatic and muscle tissues to avoid extra metabolic heat production in HS. When comparing tolerant vs. sensitive ewes in HS, liver transcriptomic changes indicated less impact of HS on tolerant ewes, which might explain why they experienced lower increment in RT under HS (Chapter 4) compared with sensitive phenotype. Overall, the obtained results confirm the relationship between heat tolerance phenotype and liver transcriptome in dairy ewes.

CHAPTER 6

Conclusions

CHAPTER 6

Conclusions

The obtained conclusions of the different experiments carried out in the present PhD thesis are mentioned below.

6.1. Specific conclusions

6.1.1. Effects of shearing strategy in Manchega and Lacaune dairy ewes

(CO = control, SBB = shorn before breeding; S100 = shorn at d 100 of pregnancy)

- Under hot summer conditions (28ºC), S100 alleviated the respiratory frequency (−37%) of pregnant ewes of either breed, when compared to SBB (shorn in spring) and CO (unshorn) ewes.
- At lambing (autumn), shorn Manchega ewes (SBB and S100) had greater glycemia (86%) than CO ewes, but no effects of shearing treatment were detected in blood metabolic indicators (insulin, BHB, NEFA) of both breeds.
- No effects of shearing treatment were detected on colostrum (composition, density, IgG and insulin) in either breed.
- No effects of shearing were detected on the weight and growth of the lambs, at birth and during suckling, in either breed.
- During suckling (autumn), Manchega S100 ewes had more milk protein (+6%) and casein (+6%) contents than CO ewes, whereas Lacaune S100 had more milk solids (+8%) and fat (+8%) contents than SBB ewes.
- No effects of shearing treatments on milk yield and composition during milking (winter and spring) were detected in both breeds.
- Although S100 ewes had greater body condition score than CO and SBB ewes at late pregnancy in both breeds, no effects of shearing treatments were detected at lambing and during lactation.

- Yet, no effects of shearing treatments were detected on body weight of Manchega ewes throughout the experiment, although Lacaune CO ewes were heavier than SBB from late-pregnancy to postlambing.
- Finally, the milk of S100 Manchega ewes showed longer rennet coagulating time (+9%) than that of CO ewes, but it was shorter (−8%) in the case of the Lacaune ewes.

6.1.2. Effects of heat stress on lactational performances and metabolic indicators according to heat tolerance phenotype in lactating Manchega ewes

Comparison of heat stress vs. thermoneutral conditions:

- Heat stressed ewes increased rectal temperature (+0.54ºC; 1.4%) and respiratory rate (+71 breaths/min; +178%).
- HS increased water consumption (+35%), reduced feed intake (−20%) and had no effects on body weight of dairy ewes.
- HS had no effects on milk yield and energy corrected milk, but reduced fat (−14%) and protein (−17%) contents in milk.
- Heat stressed ewes rose the prolactin (+415%), NEFA (+74%) and creatinine (+10%) levels in plasma, whereas they had similar glucose, insulin, and urea values.
- HS had no effects on insulin and glucose secretion kinetics during glucose tolerance test in dairy ewes.

Comparison of tolerant vs. sensitive phenotypes responses to heat stress:

- The increase in rectal temperature was lower in tolerant (+ 0.47°C) than in sensitive dairy ewes (+0.61°C) at 1700 h.
- Tolerant ewes experienced less increment in water consumption (+24%) compared with sensitive dairy ewes (+45%).
- No phenotype effects were detected on milk yield and milk composition, as well as on body weight of dairy ewes.
- No phenotype effects were detected on metabolic indicators in blood (glucose, insulin, prolactin, NEFA, creatinine, and urea) of dairy ewes.

- Finally, no major phenotype effects were detected on insulin and glucose secretion kinetics in response to glucose tolerance test. Nevertheless, tolerant ewes exhibited some degree of greater insulin resistance compared to sensitive animals.

6.1.3. Liver transcriptomics of lactating Manchega ewes according to heat tolerance phenotype under heat stress

Comparison of heat stress vs. thermoneutral conditions:

- Downregulated 39 genes (e.g., MYLPF, RYR1, MB, MYL2, SLN and TNNC1).
- Upregulated the gene ITGAD (integrin subunit alpha D), which was probably associated to cytoskeleton rearrangement of hepatocytes in response to heat stress.
- The enriched GO terms included muscle contraction, transition between fast and slow fiber, and sarcomere organization, among others.

Comparison of tolerant vs. sensitive phenotypes responses to heat stress:

- Tolerant ewes downregulated 893 genes, including those related to osteopontin protein linking to LPS and extracellular matrix (i.e., SPP1) and promoter of fatty acids oxidation as part of cytoprotective mechanisms (i.e., LEPR).
- The downregulated genes were enriched in GO terms mainly related to protein transport, extracellular exosome, cytosol, endoplasmic reticulum (ER) and protein binding.
- The downregulated pathways were endocytosis, various types of N-glycan biosynthesis, and complement and coagulation cascades.
- The downregulation of endocytosis extends the residence time of signaling receptors and transporters at the cell surface, and thereby promoting their activities in tolerant animals.
- The downregulation of N-glycan biosynthesis, and the complement and coagulation cascade pathways may indicate that tolerant ewes did not suffer major ER stress.
- Tolerant ewes upregulated 425 genes associated to fatty acid catabolism (i.e., UCP3) and involved in insulin signaling resistance (i.e., IP6K3).
- No significant enriched GO terms or KEGG pathways were detected using the upregulated genes.

6.2. General conclusions

- Shearing dairy ewes during late pregnancy is a recommended management practice since it alleviates the impact of heat stress during summer and reduces the loss of body reserves until lambing. Moreover, no negative effects on performances are expected in the subsequent lactation (e.g., colostrum composition, milk yield and composition, coagulation traits, birthweight, and growth of the lambs) and an increased milk yield may be also expected in high yielding ewes (e.g., Lacaune).

- Manchega ewes exposed to HS conditions activated mechanisms for dissipating the extra heat load (increments in water consumption, panting, and blood prolactin), reduced feed intake to avoid metabolic heat production, increased muscle catabolism (blood creatinine increase) to supply precursors (AA) for liver gluconeogenesis, and mobilized body reserves (blood NEFA increase) in late lactation. Tolerant ewes had lower rectal temperature increase at 1700 h and lower water consumption than sensitive ones, which could indicate different mechanisms involved to cope with heat stress.

- Transcriptomics supported the apparently liver-muscle crosstalk for avoiding heat production when Manchega ewes undergone HS. Overall changes in hepatic transcriptomics indicated that tolerant ewes were less impacted by HS, which might explain why they experienced lower increment in rectal temperature.

CHAPTER 7

References

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References

Abbas, Z., A. Sammad, L. Hu, H. Fang, Q. Xu, and Y. Wang. 2020. Glucose metabolism and dynamics of facilitative glucose transporters (GLUTs) under the influence of heat stress in dairy cattle. *Metabolites* 10:312. https://doi.org/10.3390/metabo10080312**.**

Abdel-Gawad, A. R., S. Hamzaoui, A. A. K. Salama, G. Caja, B. Guamis, and M. Castillo. 2012. Light backscatter evaluation of milk coagulation properties in dairy goats supplemented with soybean oil under heat stress conditions. Page 275 in Proc. XI International Conference on Goats. Book of Abstracts. Gran Canaria, ES. https://www.iga-goatworld.com/uploads/6/1/6/2/6162024/book_of_abstracts_xi_international_conference_on_goats.pdf.

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.* 10:279–287. https://doi.org/10.1016/0739-7240(93)90032-7.

Alamer, M. 2011. The role of Prolactin in thermoregulation and water balance during heat stress in domestic ruminants. *Asian J. Anim. Vet. Adv.* 6:1153–1169. https://dx.doi.org/10.3923/ajava.2011.1153.1169.

Albanell, E., P. Cáceres, G. Caja, E. Molina, and A. Gargouri. 1999. Determination of fat, protein and total solids in ovine milk by near-infrared spectroscopy. *J. AOAC Int.* 82:753–758. https://doi.org/10.1093/jaoac/82.3.753.

Albright J. L., and C. W. Alliston. 1971. Effects of varying the environment upon the performance of dairy cattle. *J. Anim. Sci.* 32:566–577. https://doi.org/10.2527/jas1971.323566x.

Aleksiev, Y. 2008. Effects of shearing on feed intake and milk yield in Tsigai ewes. *Bul. J. Agric. Sci.* 14:87−92. https://www.agrojournal.org/14/01-10-08.pdf.

Al-Haidary, A. A. 2004. Physiological responses of Naimey sheep to heat stress challenge under semi-arid environments. *Int. J. Agric. Biol.* 6:307−309. Accessed Jan. 15, 2022. https://www.researchgate.net/publication/229040471_Physiological_responses_of_Naimey_Sheep_to_heat_stress_challenge_under_semi-arid_environments.

Alhidary, I. A., S. Shini, R. A. Al Jassim, and J. B. Gaughan. 2012. Physiological responses of Australian Merino wethers exposed to high heat load. *J. Anim. Sci*. 90:212–220. https://doi.org/10.2527/jas.2011-3972.

Almanza, A., A, Carlesso, C. Chintha, S. Creedican, D. Doultsinos, B. Leuzzi, A. Luís, N. McCarthy, L. Montibeller, S. More, A. Papaioannou, F. Püschel, M.L. Sassano, J. Skoko, P. Agostinis, J. de Belleroche, L. A. Eriksson, S. Fulda, A. M. Gorman, S. Healy, A. Kozlov, C. Muñoz-Pinedo, M. Rehm, E. Chevet, and A. Samali. 2019. Endoplasmic reticulum stress signalling - from basic mechanisms to clinical applications. *FEBS J.* 286:241–278. https://doi.org/10.1111/febs.14608.

Alvarez, M. B., and H. D. Johnson. 1973. Environmental heat exposure on cattle plasma catecholamine and glucocorticoids. *J. Dairy Sci.* 56:189–194. https://doi.org/10.3168/jds.S0022-0302(73)85145-8.

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. https://doi.org/10.1007/s11033-018-4440-0.

Antonescu, C. N., T. E. McGraw, and A. Klip. 2014. Reciprocal regulation of endocytosis and metabolism. *Cold Spring Harb. Perspect. Biol.* 6: a016964. https://doi.org/10.1101/cshperspect.a016964.

AOAC. 2003. Official methods of analysis of the association of official analytical chemists. 17th ed. Association of Official Analytical Chemists, Arlington, Virginia.

Avondo, M., S. Bordonaro, D. Marletta, A. M. Guastella, and G. D'Urso. 2000. Effects of shearing and supplemental level on intake of dry ewes grazing on barley stubble. *Small Rumin. Res.* 38:237–241. https://doi.org/10.1016/s0921-4488(00)00159-0.

Bagath, M., G. Krishnan, C. Devaraj, V. P. Rashamol, P. Pragna, A. M. Lees, and V. Sejian. 2019. The impact of heat stress on the immune system in dairy cattle: A review. *Res. Vet. Sci.* 126:94−102. https://doi.org/10.1016/j.rvsc.2019.08.011.

Banchero, G. E., A. Vázquez, F. Montossi, I. de Barbieri, and G. Quintans. 2010. Pre-lambing shearing of ewes under pastoral conditions improves the early vigour of both single and twin lambs. *Anim. Prod. Sci.* 50:309−314. https://doi.org/10.1071/AN09127.

Banchero, G. E., G. Quintans, G. B. Martin, D. R. Lindsay, and J. T. B. Milton. 2004. Nutrition and colostrum production in sheep. 1. Metabolic and hormonal responses to a high-energy supplement in the final stages of pregnancy. *Reprod. Fertil. Dev.* 16:633−643. https://doi.org/10.1071/RD03091.

Bauman, D. E., and B. Currie. 1980. Partitioning of nutrients during pregnancy and lactation: A review of mechanisms involving homeostasis and homeorhesis. *J. Dairy Sci.* 63:1514−1529. https://doi.org/10.3168/jds.s0022-0302(80)83111-0.

Baumgard, L. H., and R. P. Jr. Rhoads. 2013. Effects of heat stress on postabsorptive metabolism and energetics. *Annu. Rev. Anim. Biosci.* 1:311–337. https://doi.org/10.1146/annurev-animal-031412-103644.

Beede, D. K., and R. J. Collier. 1986. Potential nutritional strategies for intensively managed cattle during thermal stress. *J. Anim. Sci.* 62:543–554. https://doi.org/10.2527/jas1986.622543.

Belhadj Slimen, I., T. Najar, A. Ghram, and M. Abdrrabba. 2016. Heat stress effects on livestock: molecular, cellular and metabolic aspects, a review. *J. Anim. Physiol. Anim. Nutr. (Berl.)* 100: 401–412. https://doi.org/10.1111/jpn.12379.

Benjamini, Y., and Y. Hochberg. 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J. R. Stat. Soc. Series B Stat. Methodol.* 57:289−300. https://doi.org/10.1111/j.2517-6161.1995.tb02031.x.

Bernabucci, U. 2019. Climate change: impact on livestock and how can we adapt. *Anim. Front.* 9:3–5. https://doi.org/10.1093/af/vfy039.

Bernabucci, U., B. Ronchi, N. Lacetera, and A. Nardone. 2002a. Markers of oxidative status in plasma and erythrocytes of transition dairy cows during hot season. *J. Dairy Sci.* 85: 2173–2179. https://doi.org/10.3168/jds.S0022-0302(02)74296-3.

Bernabucci, U., L. Basiricò, P. Morera, D. Dipasquale, A. Vitali, F. Piccioli Cappelli, and L. Calamari. 2015. Effect of summer season on milk protein fractions in Holstein cows. *J. Dairy Sci.* 98:1815−1827. https://doi.org/10.3168/jds.2014-8788.

Bernabucci, U., N. Lacetera , L. Basirico, B. Ronchi, P. Morera, E. Seren, and A. Nardone. 2006. Hot season and BCS affect leptin secretion of periparturient dairy cows. *J. Dairy Sci*. 89:348–349.

Bernabucci, U., N. Lacetera, B. Ronchi, and A. Nardone. 2002b. Effects of the hot season on milk protein fractions in Holstein cows. *Anim. Res.* 51:25–33. https://doi.org/10.1051/animres:2002006.

Bernabucci, U., N. Lacetera, L. H. Baumgard, R. P. Rhoads, B. Ronchi, and A. Nardone. 2010. Metabolic and hormonal acclimation to heat stress in domesticated ruminants. *Animal* 4:1167–1183. https://doi.org/10.1017/S175173111000090X.

Bhattacharya, A. N., and F. Hussain. 1974. Intake and utilization of nutrients in sheep fed different levels of roughage under heat stress. *J. Anim. Sci.* 38:877–886. https://doi.org/10.2527/jas1974.384877x.

Bianca, W. 1962. Relative importance of dry- and wet-bulb temperatures in causing heat stress in cattle. *Nature* 195:251–252. https://doi.org/10.1038/195251a0.

Birben, E., U. M. Sahiner, C. Sackesen, S. Erzurum, and O. Kalayci. 2012. Oxidative stress and antioxidant defense. *World Allergy Organ. J.* 5: 9–19. https://doi.org/10.1097/WOX.0b013e3182439613.

Biswas, S. K., and E. Lopez-Collazo. 2009. Endotoxin tolerance: new mechanisms, molecules and clinical significance. *Trends Immunol.* 30:475–87. https://doi.org/10.1016/j.it.2009.07.009.

Bitsikas, V., I. R. Jr. Corrêa, and B. J. Nichols. 2014. Clathrin-independent pathways do not contribute significantly to endocytic flux. *eLife* 3:e03970. https://doi.org/10.7554/eLife.03970.

Bocquier, F., F. Barillet, P. Guillouet, and M. Jacquin. 1993. Prévision de l'énergie du lait de brebis à partir de différents résultats d'analyses: proposition de lait standard pour les brebis laitières. *Ann. Zootech.* 42:57–66. https://hal.archives-ouvertes.fr/hal-00888863/document.

Bohmanova, J., I. Misztal, and J. B. Cole. 2007. Temperature-Humidity indices as indicators of milk production losses due to heat stress. *J. Dairy Sci.* 90:1947-1956. https://doi.org/10.3168/jds.2006-513.

Bouchama, A., M. A. Aziz, S. A. Mahri, M. N. Gabere, M. A. Dlamy, S. Mohammad, M. A. Abbad, and M. Hussein. 2017. A Model of exposure to extreme environmental heat uncovers the human transcriptome to heat stress. *Sci. Rep.* 7:9429. https://doi.org/10.1038/s41598-017-09819-5.

Cam, M. A., and M. Kuran. 2004. Shearing pregnant ewes to improve lamb birth weight increases milk yield of ewes and lamb weaning weight. *J. Anim. Sci.* 12:1669−1673. https://doi.org/10.5713/ajas.2004.1669.

Camara, Y., T. Mampel, J. Armengol, F. Villarroya, and L. Dejean. 2009. UCP3 expression in liver modulates gene expression and oxidative metabolism in response to fatty acids, and sensitizes mitochondria to permeability transition. *Cell. Physiol. Biochem.* 24:243–252. https://doi.org/10.1159/000233249.

Cantet, J. M., Z. Yu, and A. G. Ríus. 2021. Heat stress-mediated activation of immune-inflammatory pathways. *Antibiotics (Basel)*. 10: 1285. https://doi.org/10.3390/antibiotics10111285.

Carabaño, M. J., M. Ramón, A. Menéndez-Buxadera, A. Molina, and C. Díaz. 2019. Selecting for heat tolerance. *Anim. Front.* 9: 62–68. https://doi.org/10.1093/af/vfy033.

Carper, S. W., J. J. Duffy, and E. W. Gerner. 1987. Heat shock proteins in thermotolerance and other cellular processes. *Cancer Res.* 47: 5249–5255. Accesed Feb. 27, 2020. https://pubmed.ncbi.nlm.nih.gov/3308075/

Chauhan, S. S., P. Celi, B. J. Leury, I. J. Clarke, and F. R. Dunshea. 2014. Dietary antioxidants at supranutritional doses improve oxidative status and reduce the negative effects of heat stress in sheep. *J. Anim. Sci.* 92:3364–3374. https://doi.org/10.2527/jas.2014-7714.

Cheatham, B., A. Volchuk, C. R. Kahn, L. Wang, C. J. Rhodes, and A. Klip. 1996. Insulin-stimulated translocation of GLUT4 glucose transporters requires SNARE-complex proteins. *Proc. Natl. Acad. Sci. U. S. A.* 93:15169–15173. https://doi.org/10.1073/pnas.93.26.15169.

Close, W. H., L. E. Mount, and I. B. Start. 1971.The influence of environmental temperature and plane of nutrition on heat losses from groups of growing pigs. *Anim. Prod.* 13:285–294. https://doi.org/10.1017/S0003356100029731.

Cnop, M., F. Foufelle, and L. A. Velloso. 2012. Endoplasmic reticulum stress, obesity and diabetes. *Trends Mol. Med.* 18:59–68. https://doi.org/10.1016/j.molmed.2011.07.010.

Collier R. J., J. L. Collier, R. P. Rhoads, and L. H. Baumgard. 2008. Invited review: Genes involved in the Bovine heat stress response. *J. Dairy Sci.* 91: 445−454. https://doi.org/10.3168/jds.2007-0540.

Collier, R. J., and K. G. Gebremedhin. 2015. Thermal biology of domestic animals. *Annu. Rev. Anim. Biosci.* 3:513–532. https://doi.org/10.1146/annurev-animal-022114-110659.

Collier, R. J., D. K. Beede, W. W. Thatcher, L. A. Israel, and C. J. Wilcox. 1982a. Influences of environment and its modification on dairy animal health and production. *J. Dairy Sci.* 65:2213–2227. https://doi.org/10.3168/jds.S0022-0302(82)82484-3.

Collier, R. J., G. E. Dahl, and M. J. VanBaale. 2006. Major Advances Associated with Environmental Effects on Dairy Cattle. *J. Dairy Sci.* 89:1244-1253. https://doi.org/10.3168/jds.S0022-0302(06)72193-2.

Collier, R. J., L. H. Baumgard, R. B. Zimbelman, and Y. Xiao. 2018. Heat stress: physiology of acclimation and adaptation. *Anim. Front.* 9:12–19. https://doi.org/10.1093/af/vfy031.

Collier, R. J., R. M. Eley, A. K. Sharma, R. M. Pereira, and D. E. Buffington. 1981. Shade management in subtropical environment for milk yield and composition in Holstein and Jersey cows. *J. Dairy Sci.* 64:844–849. https://doi.org/10.3168/jds.S0022-0302(81)82656-2.

Collier, R. J., S. G. Doelger, H. H. Head, W. W. Thatcher, and C. J. Wilcox. 1982b. Effects of heat stress during pregnancy on maternal hormone concentrations, calf birth weight and postpartum milk yield of Holstein cows. *J. Anim. Sci.* 54:309–319. https://doi.org/10.2527/jas1982.542309x.

Conesa, A., P. Madrigal, S. Tarazona, D. Gomez-Cabrero, A. Cervera, A. McPherson, M. W. Szcześniak, D.J. Gaffney, L.L. Elo, X. Zhang, and A. Mortazavi. 2016. A survey of best practices for RNA-seq data analysis. *Genome Biol.* 17:13. https://doi.org/10.1186/s13059-016-0881-8.

Contreras-Jodar, A., A. A. K. Salama, S. Hamzaoui, M. Vailati-Riboni, G. Caja, and J. J. Loor. 2018. Effects of chronic heat stress on lactational performance and the transcriptomic profile of blood cells in lactating dairy goats. *J. Dairy Res.* 85:423–430. https://doi.org/10.1017/S0022029918000705.

Contreras-Jodar, A., N. H. Nayan, S. Hamzaoui, G. Caja, and A. A. K. Salama. 2019. Heat stress modifies the lactational performances and the urinary metabolomic profile related to gastrointestinal microbiota of dairy goats. *PLoS One* 14:e0202457. https://doi.org/10.1371/journal.pone.0202457.

Cossins A., J. Fraser, M. Hughes, and A. Gracey. 2006. Post-genomic approaches to understanding the mechanisms of environmentally induced phenotypic plasticity. *J. Exp. Biol.* 209:2328–2336. https://doi.org/10.1242/jeb.02256.

Crilly, J. P., C. Phythian, and M. Evans. 2021. Advances in managing pregnancy toxaemia in sheep. *In Practice.* 43:79–94. https://doi.org/10.1002/inpr.17.

Dabiri N., S. T. Morris, M. Wallentine, S. N. Mccutcheon, W. J. Parker, and G. A. Wickham. 1996. Effects of pre-lamb shearing on feed intake and associated productivity of May- and August-lambing ewes. *NZ J. Agric. Res.* 39:53-62. https://doi.org/10.1080/00288233.1996.9513162.

Dahl, G. E., S. Tao, and J. Laporta. 2020. Heat stress impacts immune status in cows across the life cycle. *Front. Vet. Sci.* 7:116. https://doi.org/10.3389/fvets.2020.00116.

Dalle-Donne, I., R. Rossi, A. Milzani, P. Di Simplicio, and R. Colombo. 2001. The actin cytoskeleton response to oxidants: from small heat shock protein phosphorylation to changes in the redox state of actin itself. *Free Radic. Biol. Med.* 31:1624–1632. https://doi.org/10.1016/s0891-5849(01)00749-3.

Dangi, S. S., M. Gupta, D. Maurya, V. P. Yadav, R. P. Panda, G. Singh, N. H. Mohan, S. K. Bhure, B. C. Das, S. Bag, R. Mahapatra, G. Taru Sharma, and M. Sarkar. 2012. Expression profile of HSP genes during different seasons in goats (Capra hircus). *Trop. Anim. Health Prod.* 44:1905–1912. https://doi.org/10.1007/s11250-012-0155-8.

De Abreu, A. S., V. Fischer, A. Thaler, M. T. Stumpf, F. Petronilho, D. S. Florentino, N. R. Hlavac, M. Uczay, E. Paludo, P. H. E. Weiss, and C. I. G. Vogel. 2020. Access to shade reduces DNA damage of Holstein cows under mild heat stress. *Anim. Prod. Sci.* 60:1539-1546. https://doi.org/10.1071/AN19075.

Dikmen S., and P. J. Hansen. 2009. Is the temperature-humidity index the best indicator of heat stress in lactating dairy cows in a subtropical environment? *J. Dairy Sci.* 92:109-116, https://doi.org/10.3168/jds.2008-1370.

Do Amaral, B. C., E. E. Connor, S. Tao, J. Hayen, J. Bubolz, and G. E. Dahl. 2010. Heat stress abatement during the dry period influences prolactin signaling in lymphocytes. *Domest. Anim. Endocrinol.* 38:38–45. APA https://doi.org/10.1016/j.domaniend.2009.07.005.

Doney, J. M., J. N. Peart, W. F. Smith, and F. Louda. 1979. A consideration of the techniques for estimation of milk yield by suckled sheep and a comparison of estimates obtained by

two methods in relation to the effect of breed, level of production and the stage of lactation. *J. Agric. Sci. Camb.* 92:123–132. https://doi.org/10.1017/S0021859600060573.

Dyrmundsson, O.R. 1991. Shearing time of sheep with special reference to conditions in northern Europe: a review. *Icel. Agric. Sci.* 5:39−46. https://ias.is/buvisindi-5-1991/

Ehrhardt, R. 2021. The benefits of shearing before lambing. MSU Extension. Michigan State University. Available at https://www.canr.msu.edu/sheep_goats/reproduction/the-benefits-of-shearing-before-lambing . Accessed on: 30 July 2021.

Einspanier, R., and D. Schams. 1991. Changes in concentrations of insulin-like growth factor 1, insulin and growth hormone in bovine mammary gland secretion ante and post partum. *J. Dairy Res.* 58:171–178. https://doi.org/10.1017/S002202990002971X.

Elhadi, A., A. A. K. Salama, X. Such, E. Albanell, P. G. Toral, G. Hervás, P. Frutos, and G. Caja. 2019. Effects of shearing 2 breeds of dairy ewes during lactation under mild winter conditions. *J. Dairy Sci.* 102:1712–1724. https://doi.org/10.3168/jds.2018-15380.

Elhadi, A., and G. Caja. 2018. Thermo-physiological traits within a flock of dairy ewes and variability in the response to a heat stress challenge. ADSA Annual Meeting. Knoxville, TN. *J Dairy Sci.* 101:165 https://www.adsa.org/Meetings/Past-Meetings/2018.

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. https://doi.org/10.1007/s00484-019-01677-2.

England, K., C. O'Driscoll, and T. G. Cotter. 2004. Carbonylation of glycolytic proteins is a key response to drug-induced oxidative stress and apoptosis. *Cell Death Differ.* 11: 252–260. https://doi.org/10.1038/sj.cdd.4401338.

Etherton T. D., and C. M. Evock. 1986. Stimulation of lipogenesis in bovine adipose tissue by insulin and insulin-like growth factor. *J. Anim. Sci.* 62:357−362. https://doi.org/10.2527/jas1986.622357x.

FAOSTAT. 2020. Food and Agriculture Organization of the United Nations. Statistics database. Accesed April 2, 2022. https://www.fao.org/faostat/en/#data.

Fielder, S. E. 2021. Serum biochemical reference ranges. MSD Manual, veterinary manual. Available at https://www.msdvetmanual.com/special-subjects/reference-guides/serum-biochemical-reference-ranges . Accessed on 30 July 2021.

Finochiaro, R., J. B. C. H. M. Van Kaam, B. Portolano, and I. Miztal. 2005. Effect of heat stress on production of Mediterranean dairy sheep. *J. Dairy Sci.* 88:1855–1864. https://doi.org/10.3168/jds.S0022-0302(05)72860-5.

Flamment, M., E. Hajduch, P. Ferré, and F. Foufelle. 2012. New insights into ER stress-induced insulin resistance. *Trends Endocrinol. Metab.* 23:381–390. https://doi.org/10.1016/j.tem.2012.06.003.

Flores, C., G. Caja, R. Casals, E. Albanell, and X. Such. 2008. Performance of dairy ewes fed diets with a fibrolytic enzyme product included in the concentrate during the suckling period. *Animal* 2:962-968. https://doi.org/10.1017/S1751731108001961.

Fulda, S., A. M. Gorman, O. Hori, and A. Samali. 2010. Cellular stress responses: cell survival and cell death. *Int. J. Cell Biol.* 2010:214074. https://doi.org/10.1155/2010/214074.

Galán, E., P. Llonch, A. Villagrá, H. Levit, S. Pinto, and A. Del Prado. 2018. A systematic review of non-productivity-related animal-based indicators of heat stress resilience in dairy cattle. *PloS One* 13:e0206520. https://doi.org/10.1371/journal.pone.0206520.

Gallego, R., O. García, R. Arias, M. D. Pérez-Guzmán, and V. Montoro. 2016. Evolución de la mejora genética en la raza ovina Manchega variedad blanca. Efectos de la participación en el programa de selección. *Archivos de Zootecnia* 65:437–440. Accessed Apr. 13, 2022. https://www.redalyc.org/articulo.oa?id=49549092026.

Gao, J., Q. Li, and D. Li. 2021. Novel proteome and N-Glycoproteome of the thermophilic fungus chaetomium thermophilum in response to high temperature. *Front. Microbiol.* 12:644984. https://doi.org/10.3389/fmicb.2021.644984.

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 https://doi.org/10.3168/jds.2016-11913.

Gao, S. T., L. Ma, Z. Zhou, Z. K. Zhou, L. H. Baumgard, D. Jiang, M. Bionaz, and D. P. Bu. 2019. Heat stress negatively affects the transcriptome related to overall metabolism and milk protein synthesis in mammary tissue of midlactating dairy cows. *Physiol. Genomics* 51:400–409. https://doi.org/10.1152/physiolgenomics.00039.2019.

García-Rodríguez, A., I. Beltrán de Heredia, J. Arranz, L. M. Oregui, and R. Ruíz. 2012. Effects of winter shearing on reproductive and lactation performances of the Latxa dairy ewe. Pages 131−134 in New Trends for Innovation in the Mediterranean Animal Production. EAAP – European Federation of Animal Science, No. 129. R. Bouche, A. Derkimba, and F. Casabianca, ed. Wageningen Academic Publishers, Wageningen, NL. https://doi.org/10.3920/978-90-8686-726-4_18.

Garner J. B., M. Douglas, S. R. O. Williams, W. J. Wales, L. C. Marett, K. DiGiacomo, B. J. Leury, and B. J. Hayes . 2017. Responses of dairy cows to short-term heat stress in controlled-climate chambers. *Anim. Prod.* Sci. 57:1233–1241. https://doi.org/10.1071/AN16472.

Garner, J. B., A. J. Chamberlain, C. Vander Jagt, T. T. T. Nguyen, B. A. Mason, L. C. Marrett, B. J. Leury, W. J. Wales, and B. J. Hayes. 2020. Gene expression of the heat stress response in bovine peripheral white blood cells and milk somatic cells in vivo. *Sci. Rep.* 10:19181. https://doi.org/10.1038/s41598-020-75438-2.

Garrett, A. T., N. G. Goosens, N. J. Rehrer, M. J. Patterson, and J. D. Cotter. 2009. Induction and decay of short-term heat acclimation. *Eur. J. Appl. Physiol.* 107:659–670. https://doi.org/10.1007/s00421-009-1182-7.

Gaughan, J., N. Lacetera, S. E. Valtorta, H. H. Khalifa, L. Hahn, and T. Mader. 2009. Response of domestic animals to climate challenges. Pages 131–170 in Biometeorology for

Adaptation to Climate Variability and Change. K. L. Ebi, I. Burton, and G. R. McGregor, ed. Springer, Dordrecht, NL. https://doi.org/10.1007/978-1-4020-8921-3_7.

Gracey, A. Y. 2007. Interpreting physiological responses to environmental change through gene expression profiling. *J. Exp. Biol.* 210:1584–1592. https://doi.org/10.1242/jeb.004333.

Guerriero, V. Jr., and D. A. Raynes. 1990. Synthesis of heat stress proteins in lymphocytes from livestock. *J. Anim. Sci.* 68:2779–2783. https://doi.org/10.2527/1990.6892779x.

Guo, Z., S. Gao, J. Ouyang, L. Ma, and D. Bu. 2021. Impacts of Heat Stress-Induced Oxidative Stress on the Milk Protein Biosynthesis of Dairy Cows. *Animals* 11:726. https://doi.org/10.3390/ani11030726.

Haire, A., J. Bai, X. Zhao, Y. Song, G. Zhao, A. Dilixiati, J. Li, W. Q. Sun, P. Wan, X. Fu, and A. Wusiman. 2022. Identifying the heat resistant genes by multi-tissue transcriptome sequencing analysis in Turpan Black sheep. *Theriogenology* 179:78–86. https://doi.org/10.1016/j.theriogenology.2021.11.008.

Hales, J. R. S., and G. D. Brown. 1974. Net energetic and thermoregulatory efficiency during panting in the sheep. *Comp. Biochem. Physiol. A. Physiol.* 49:413–422. https://doi.org/10.1016/0300-9629(74)90557-X.

Hammami, H., J. Bormann, N. M'hamdi, H. H. Montaldo, and N. Gengler. 2013. Evaluation of heat stress effects on production traits and somatic cell score of Holsteins in a temperate environment. *J. Dairy Sci.* 96:1844–1855. https://doi.org/10.3168/jds.2012-5947.

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

Hassan, F. U., A. Nawaz, M. S. Rehman, M. A. Ali, S. M. R. Dilshad, and C. Yang. 2019. Prospects of HSP70 as a genetic marker for thermo-tolerance and immuno-modulation in animals under climate change scenario. *Anim. Nutr.* 5:340–350. https://doi.org/10.1016/j.aninu.2019.06.005.

Hetz, C., F. Martinon, D. Rodriguez, and L. H. Glimcher. 2011. The unfolded protein response: integrating stress signals through the stress sensor IRE1α. *Physiol. Rev.* 91:1219–1243. https://doi.org/10.1152/physrev.00001.2011.

Hill, T. G., and C. W. Alliston. 1981. Effects of thermal stress on plasma concentrations of luteinizing hormone, progesterone, prolactin and testosterone in the cycling ewe. *Theriogenology* 15:201–209. https://doi.org/10.1016/s0093-691x(81)80008-8.

Hoene, M., and C. Weigert. 2010. The stress response of the liver to physical exercise. *Exerc. Immunol. Rev.* 16:163–183. https://pubmed.ncbi.nlm.nih.gov/20839498.

Homma, T., and J. Fujii. 2016. Heat stress promotes the down-regulation of IRE1α in cells: An atypical modulation of the UPR pathway. *Exp. Cell Res.* 349:128–138. https://doi.org/10.1016/j.yexcr.2016.10.006.

Hooley, R. D., J. K. Findlay, and R. G. Stephenson. 1979. Effect of heat stress on plasma concentrations of prolactin and luteinizing hormone in ewes. *Aust. J. Biol. Sci.* 32:231–235. https://doi.org/10.1071/bi9790231.

Horowitz, M. 2002. From molecular and cellular to integrative heat defense during exposure to chronic heat. *Comp. Biochem. Physiol. A. Mol. Integr. Physiol.* 131:475–483. https://doi.org/10.1016/s1095-6433(01)00500-1.

Indu, S., V. Sejian, and S. M. K. Naqvi. 2014. Impact of simulated semiarid tropical environmental conditions on growth, physiological adaptability, blood metabolites and endocrine responses in Malpura ewes. *Anim. Prod. Sci.* 55:766–776. https://doi.org/10.1071/AN14085.

INRA. 2018. Institut National de la Recherche Agronomique. INRA feeding system for ruminants. ed. Wageningen Academic Publishers, Wageningen, NL.

IPCC. 2014. Climate Change 2014: Synthesis Report. Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Core Writing Team, R.K. Pachauri and L.A. Meyer (eds.)]. IPCC, Geneva, Switzerland, 151 pp.

Itoh, F., Y. Obara, M. T. Rose, H. Fuse, and H. Hashimot. 1998. Insulin and glucagon secretion in lactating cows during heat exposure. *J. Anim. Sci.* 76:2182–2189. http://dx.doi.org/10.2527/1998.7682182x.

Jiang Y., Z. Tao, H. Chen, and S. Xia. 2021. Endoplasmic reticulum quality control in immune cells. *Front. Cell. Dev. Biol.* 9:740653 https://doi.org/10.3389/fcell.2021.740653.

Jiao, D., K. Ji, H. Liu, W. Wang, X. Wu, J. Zhou, Y. Zhang, H. Zhou, J. Hickford, A. A. Degen, and G. Yang. 2021. Transcriptome analysis reveals genes involved in thermogenesis in two cold-exposed sheep breeds. *Genes* 12:375. https://doi.org/10.3390/genes12030375.

Johnson, H. D., and W. J. Vanjonack. 1976. Effects of environmental and other stressors on blood hormone patterns in lactating animals. *J. Dairy Sci.* 59:1603–1617. https://doi.org/10.3168/jds.S0022-0302(76)84413-X.

Joy, A., F. R. Dunshea, B. J. Leury, I. J. Clarke, K. DiGiacomo, and S. S. Chauhan. 2020. Resilience of small ruminants to climate change and increased environmental temperature: A review. *Animals* 10:867. https://doi.org/10.3390/ani10050867.

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. https://doi.org/10.1016/S0301-6226(01)00330-X.

Kahl, S., T. H. Elsasser, R. P. Rhoads, R. J. Collier, and L. H. Baumgard. 2015. Environmental heat stress modulates thyroid status and its response to repeated endotoxin challenge in steers. *Domest. Anim. Endocrinol.* 52:43–50. https://doi.org/10.1016/j.domaniend.2015.02.001.

Katz, A., S. S. Nambi, K. Mather, A. D. Baron, D. A. Follmann, G. Sullivan, and M. J. Quon. 2000. Quantitative insulin sensitivity check index: a simple, accurate method for assessing insulin sensitivity in humans. *J. Clin. Endocrinol. Metab.* 85:2402–2410. https://doi.org/10.1210/jcem.85.7.6661.

Kenyon, P. R, S. K. Maloney, and D. Blache. 2014. Review of sheep body condition score in relation to production characteristics. *NZ. J. Agric. Res.* 57:38−64. https://doi.org/10.1080/00288233.2013.857698.

Kenyon, P. R., S. T. Morris, D. K. Revell, and S. N. McCutcheon. 2003. Shearing during pregnancy -review of a policy to increase birthweight and survival of lambs in New Zealand pastoral farming systems. *NZ. Vet. J.* 51:200−207. http://dx.doi.org/10.1080/00480169.2003.36368.

Kerestes, M., V. Faigl, M. Kulcsár, O. Balogh, J. Földi, H. Fébel, Y. Chilliard, and G. Huszenicza. 2009. Periparturient insulin secretion and whole-body insulin responsiveness in dairy cows showing various forms of ketone pattern with or without puerperal metritis. *Domest. Anim. Endocrinol.* 37:250–261. https://doi.org/10.1016/j.domaniend.2009.07.003.

Kessler E. C., R. M. Bruckmaier, and J. J. Gross. 2019. Immunoglobulin G content and colostrum composition of different goat and sheep breeds in Switzerland and Germany. *J. Dairy Sci.* 102:5542−5549. https://doi.org/10.3168/jds.2018-16235.

Kiang, J. G., and G. C. Tsokos. 1998. Heat shock protein 70 kDa: molecular biology, biochemistry, and physiology. *Pharmacol. Ther.* 80:183–201. https://doi.org/10.1016/s0163-7258(98)00028-x.

Kitajima, K., K. Oishi, M. Miwa, H. Anzai, A. Setoguchi, Y. Yasunaka, Y. Himeno, H. Kumagai, and H. Hirooka. 2021. Effects of heat stress on heart rate variability in free-moving sheep and goats assessed with correction for physical activity. *Front. Vet. Sci.* 8:658763. https://doi.org/10.3389/fvets.2021.658763.

Knight, T. W., R. Bencini, N. A. Haack, and A. F. Death. 1993. Effects of shearing on milk yields and milk composition in machine-milked Dorset ewes. *NZ J. Agric. Res.* 36:123−132. http://dx.doi.org/10.1080/00288233.1993.10427493.

Koch, F., O. Lamp, M. Eslamizad, J. Weitzel, and B. Kuhla. 2016. Metabolic Response to Heat stress in late-pregnant and early lactation dairy cows: Implications to liver-muscle crosstalk. *PLoS One* 11:e0160912. https://doi.org/10.1371/journal.pone.0160912.

Koch, F., U. Thom, E. Albrecht, R. Weikard, W. Nolte, B. Kuhla, and C. Kuehn. 2019. Heat stress directly impairs gut integrity and recruits distinct immune cell populations into the bovine intestine. *Agric. Sci.* 116:10333−10338. https://doi.org/10.1073/pnas.1820130116.

Kubes, P., and C. Jenne. 2018. Immune responses in the liver. **Annu. Rev. Immunol.** 36:247–277. https://doi.org/10.1146/annurev-immunol-051116-052415.

Lacetera, N. 2019. Impact of climate change on animal health and welfare. *Anim. Front.* 9:26–31. https://doi.org/10.1093/af/vfy030.

Lacetera, N., U. Bernabucci, D. Scalia, L. Basiricò, P. Morera, and A. Nardone. 2006. Heat stress elicits different responses in peripheral blood mononuclear cells from Brown Swiss and Holstein cows. *J. Dairy Sci.* 89: 4606–4612. https://doi.org/10.3168/jds.S0022-0302(06)72510-3.

Lan, X., J. C. Hsieh, C. J. Schmidt, Q. Zhu, and S. J. Lamont. 2016. Liver transcriptome response to hyperthermic stress in three distinct chicken lines. *BMC Genomics* 17:955. https://doi.org/10.1186/s12864-016-3291-0.

Lanks, K. W. 1986. Modulators of the eukaryotic heat shock response. *Exp. Cell Res.* 165:1–10. https://doi.org/10.1016/0014-4827(86)90528-8.

Lecchi, C., N. Rota, A. Vitali, F. Ceciliani, and N. Lacetera. 2016. In vitro assessment of the effects of temperature on phagocytosis, reactive oxygen species production and apoptosis in bovine polymorphonuclear cells. *Vet. Immunol. Immunopathol.* 182:89–94. https://doi.org/10.1016/j.vetimm.2016.10.007.

Leibovich, H., A. Zenou, P. Seada, and J. Miron. 2011. Effects of shearing, ambient cooling and feeding with byproducts as partial roughage replacement on milk yield and composition in Assaf sheep under heat-load conditions. *Small Rumin. Res.* 99:153−159. https://doi.org/10.1016/j.smallrumres.2011.03.049.

Li, H., Y. Zhang , R. Li , Y. Wu, D. Zhang, H. Xu, Y. Zhang, Z. Qi. 2021. Effect of seasonal thermal stress on oxidative status, immune response and stress hormones of lactating dairy cows. *Anim. Nutr.* 7:216-223. https://doi.org/10.1016/j.aninu.2020.07.006.

Li, Y., L. Kong, M. Deng, Z. Lian, Y. Han, B. Sun, Y. Guo, G. Liu, and D. Liu. 2019. Heat stress-responsive transcriptome analysis in the liver tissue of Hu sheep. *Genes (Basel)* 10:395. https://doi.org/10.3390/genes10050395.

Lin, J. H., P. Walter, and T. S. Yen. 2008. Endoplasmic reticulum stress in disease pathogenesis. *Annu. Rev. Pathol.* 3:399–425. https://doi.org/10.1146/annurev.pathmechdis.3.121806.151434.

Lindquist, S. 1986. The heat-shock response. *Annu. Rev. Biochem.* 55:1151–1191. https://doi.org/10.1146/annurev.bi.55.070186.005443.

Liu, S., T. Yue, M. J. Ahmad, X. Hu, X. Zhang, T. Deng, Y. Hu, C. He, Y. Zhou, and L. Yang. 2020. Transcriptome analysis reveals potential regulatory genes related to heat tolerance in Holstein dairy cattle. *Genes* 11:68. https://doi.org/10.3390/genes11010068.

Liu, X., and R. M. Green. 2019. Endoplasmic reticulum stress and liver diseases. *Liver Res.* 3:55–64. https://doi.org/10.1016/j.livres.2019.01.002.

López-Hernández, T., V. Haucke, and T. Maritzen. 2020. Endocytosis in the adaptation to cellular stress. *Cell Stress* 4:230–247. https://doi.org/10.15698/cst2020.10.232.

Lowe, R., N. Shirley, M. Bleackley, S. Dolan, and T. Shafee. 2017. Transcriptomics technologies. *PLoS Comput. Biol.* 13:e1005457. https://doi.org/10.1371/journal.pcbi.1005457.

Lu, C. D. 1989. Effects of heat stress on goat production. *Small Rumin. Res.* 2:151-162. https://doi.org/10.1016/0921-4488(89)90040-0.

Lu, Q., J. Wen, and H. Zhang. 2007. Effect of chronic heat exposure on fat deposition and meat quality in two genetic types of chicken. *Poult. Sci*. 86:1059–1064. https://doi.org/10.1093/ps/86.6.1059.

Lu, Z., M. Chu, Q. Li, M. Jin, X. Fei, L. Ma, L. Zhang, and C. Wei. 2019. Transcriptomic Analysis Provides Novel Insights into Heat Stress Responses in Sheep. *Animals (Basel)* 9:387. https://doi.org/10.3390/ani9060387.

Ma, Y., and L. M. Hendershot. 2004. ER chaperone functions during normal and stress conditions. *J. Chem. Neuroanat.* 28: 51–65. https://doi.org/10.1016/j.jchemneu.2003.08.007.

Mader, T. L., M. S. Davis, and T. Brown-Brandl. 2006. Environmental factors influencing heat stress in feedlot cattle. *J. Anim. Sci.* 84:712–719. https://doi.org/10.2527/2006.843712x.

MAPA. 2007. Ministerio de Agricultura, Pesca y Alimentación. Guías de prácticas correctas de higiene Ovino de leche. http://www.mapama.gob.es/es/ganaderia/publicaciones/CCAE_tcm30-105306.pdf. Accessed Oct. 27, 2020.

Marai, I. F. M., A. A. El-Darawanya, A. Fadiel, and M. A. M. Abdel-Hafez. 2007. Physiological traits as affected by heat stress in sheep – a review. *Small Rumin. Res.* 71:1–12 https://doi.org/10.1016/j.smallrumres.2006.10.003.

Marra, F., A. Gastaldelli, G. Svegliati Baroni, G. Tell, and C. Tiribelli. 2008. Molecular basis and mechanisms of progression of non-alcoholic steatohepatitis. *Trends. Mol. Med.* 14:72–81. https://doi.org/10.1016/j.molmed.2007.12.003.

McGuire, M. A., D. K. Beede, R. J. Collier, F. C. Buonomo, M. A. DeLorenzo, C. J. Wilcox, G. B. Huntington, and C. K. Reynolds. 1991. Effects of acute thermal stress and amount of feed intake on concentrations of somatotropin, insulin-like growth factor (IGF)-I and IGF-II, and thyroid hormones in plasma of lactating Holstein cows. *J. Anim Sci.* 69:2050–2056. https://doi.org/10.2527/1991.6952050x.

McManus, C. M., D. A. Faria, C. M. Lucci, H. Louvandini, S. A. Pereira, and S. R. Paiva. 2020. Heat stress effects on sheep: Are hair sheep more heat resistant? *Theriogenology* 155:157–167. https://doi.org/10.1016/j.theriogenology.2020.05.047.

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. https://doi.org/10.3390/ani9080567.

Mehaba, N., W. Coloma-Garcia, X. Such, G. Caja, and A. A. K. Salama. 2021. Heat stress affects some physiological and productive variables and alters metabolism in dairy ewes. *J. Dairy Sci*. 104:1099–1110. https://doi.org/10.3168/jds.2020-18943.

Meyer, U., M. Everinghoff , D. Gädeken, and G. Flachowsky. 2004. Investigations on the water intake of lactating dairy cows. *Livest. Prod. Sci.* 90: 117-121. https://doi.org/10.1016/j.livprodsci.2004.03.005.

Miao, Q. X., X. Y. Si, Y. J. Xie, L. Chen, X. F. Tang, and H. F. Zhang. 2022. Acute heat stress alters the expression of genes and proteins associated with the unfolded protein response pathway in the liver of broilers. *Br. Poult. Sci.* 63:125–132. https://doi.org/10.1080/00071668.2021.1969644.

Milán, M. J., F. Frendi, R. González-González, and G. Caja. 2014. Cost structure and profitability of Assaf dairy sheep farms in Spain. *J. Dairy Sci.* 97:5239–5249. https://doi.org/10.3168/jds.2013-7884.

Moallen, U., A. Rozov, E. Gootwine, and H. Honig. 2012. Plasma concentrations of key metabolites and insulin in late-pregnant ewes carrying 1 to 5 fetuses. *J. Anim. Sci.* 90:318−324. https://doi.org/10.2527/jas.2011-3905.

Mohr, E., and H. Krzywanek. 1990. Variations of core-temperature rhythms in unrestrained sheep. *Physiol. Behav.* 48:467–473. https://doi.org/10.1016/0031-9384(90)90345-5.

Monty, D. E., L. M. Kelley, and W. R. Rice. 1991. Acclimatization of St.-Croix, Karakul and Rambouillet sheep to intense and dry summer heat. *Small Rumin. Res.* 4:379–392. https://doi.org/10.1016/0921-4488(91)90083-3.

Morris, S. T., and S. N. McCutcheon. 1997. Selective enhancement of growth in twin foetuses by shearing ewes in early gestation. *Anim. Sci.* 65:105−110. https://doi.org/10.1017/S1357729800016350.

Mukherjee, S., J. Haubner, and A. Chakraborty. 2020. Targeting the inositol pyrophosphate biosynthetic enzymes in metabolic diseases. *Molecules* 25:1403. https://doi.org/10.3390/molecules25061403.

Mullan, W. M. A. 2008. Determination of the theoretical yield of cheddar cheese using milk composition only and a modified version of the van slyke yield equation. Available at https://www.dairyscience.info/newCalculators/yield-01.asp. Accessed: 17 November 2020.

Nardone, A., N. Lacetera, U. Bernabucci, and B. Ronchi. 1997. Composition of colostrum from dairy heifers exposed to high air temperatures during late pregnancy and the early postpartum period. *J. Dairy Sci.* 80:838–844. https://doi.org/10.3168/jds.S0022-0302(97)76005-3.

Nieddu, G., and G. Caja. 2017. Eficiencia de identificación electrónica y recogida de datos de leche en un sistema automático para ovejas y cabras lecheras. Pages 33–35 in Proc. XVII Jornadas de Producción Animal de AIDA (Asociación Interprofesional para el Desarrollo Agrario), Zaragoza, Spain.

Noordhuizen, J., and J. M. Bonnefoy. 2015. Heat stress in dairy cattle: major effects and practical management measures for prevention and control. *SOJ Vet. Sci.* 1:1–7. http://dx.doi.org/10.15226/2381-2907/1/1/00103.

Nowak, J., J. Olszewski, and E. Kozal. 1994. Insulin and sugar concentration changes in mammary secretion in sheep during the periparturient period. *Reprod. Nutr. Dev.* 34:3−8. https://doi.org/10.1051/rnd:19940101.

NRC. 1971. National Research Council. A Guide to Environmental Research on Animal. ed. National Academies Press, Washington, DC.

NRC. 1981. National Research Council. Effect of Environment on Nutrient Requirements of Domestic Animals. ed. National Academies Press, Washington, DC. https://www.ncbi.nlm.nih.gov/books/NBK232324/

NRC. 2001. National Research Council. Nutrient Requirements of Dairy Cattle. ed. National Academies Press, Washington, DC. https://doi.org/10.17226/9825.

Ozawa, T., M. Tanaka, H. Suzuki, and M. Nishikimi. 1987. Structure and function of mitochondria: Their organization and disorders. *Brain Dev.* 9:76–81. https://doi.org/10.1016/S0387-7604(87)80021-9.

Pant, M., N. C. Bal, and M. Periasamy. 2016. Sarcolipin: a key thermogenic and metabolic regulator in skeletal muscle. *Trends Endocrinol. Metab. 27*:881–892. https://doi.org/10.1016/j.tem.2016.08.006.

Peana, I., G. Fois, and A. Cannas. 2007. Effects of heat stress and diet on milk production and feed and energy intake of Sarda ewes. *Ital. J. Anim. Sci.* 6:577–579. https://doi.org/10.4081/ijas.2007.1s.577.

Pellegrini, O., F. Remeuf, M. Rivemale, and F. Barillet. 1997. Renneting properties of milk from individual ewes: influence of genetic and non-genetic variables, and relationship with physicochemical characteristics. *J. Dairy Res.* 64:355−366. https://doi.org/10.1017/S0022029997002203.

Polyzos, S. A., J. Kountouras, and C. S. Mantzoros. 2015. Leptin in nonalcoholic fatty liver disease: a narrative review. *Metabolism* 64:60–78. https://doi.org/10.1016/j.metabol.2014.10.012.

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. (Berl)* 102:825–836. https://doi.org/10.1111/jpn.12892.

Pulina, G., M. J. Milán, M. P. Lavín, A. Theodoridis, E. Morin, J. Capote, D. L. Thomas, A. H. D. Francesconi, and G. Caja. 2018. Invited review: Current production trends, farm structures, and economics of the dairy sheep and goat sectors. *J. Dairy Sci.* 101:6715–6729. https://doi.org/10.3168/jds.2017-14015.

Qaid, M. M., and M. M. Abdelrahman. 2016. Role of insulin and other related hormones in energy metabolism. A review. *Cogent Food Agric.* 2:1267691. https://doi.org/10.1080/23311932.2016.1267691.

R Core Team. 2020. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Ramón, M., C. Diaz, M. D. Perez-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. https://doi .org/10.3168/jds.2016-10909.

Ravagnolo O., and I. Misztal. 2000. Genetic Component of Heat Stress in Dairy Cattle, Parameter Estimation. *J. Dairy Sci.* 83:2126-2130. https://doi.org/10.3168/jds.S0022-0302(00)75095-8.

Reintke J., K. Brügemann, T. Yin, H. Wagner, A. Wehrend, A. Müller, and S. König. 2021. Associations between minerals and metabolic indicators in maternal blood pre- and postpartum with ewe body condition, methane emissions, and lamb body weight development. *Animal* 15:100034. https://doi.org/10.1016/j.animal.2020.100034.

Renaudeau, D., A. Collin, S. Yahav, V. de Basilio, J. L. Gourdine, and R. J. Collier. 2012. Adaptation to hot climate and strategies to alleviate heat stress in livestock production. *Animal* 6:707–728. https://doi.org/10.1017/S1751731111002448.

Revell, D. K., S. F. Main, B. H. Breier, Y. H. Cottam, M. Hennies, and S. N. McCutcheon. 2000. Metabolic responses to mid-pregnancy shearing that are associated with a selective increase in the birth weight of twin lambs. *Domest. Anim. Endocrinol.* 18:409–422. https://doi.org/10.1016/S0739-7240(00)00059-X.

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. https://doi.org/10.3168/jds.2008-1641.

Rhoads, R. P., A. J. La Noce, J. B. Wheelock, and L. H. Baumgard. 2011. Alterations in expression of gluconeogenic genes during heat stress and exogenous bovine somatotropin administration. *J. Dairy Sci.* 94:1917–1921. https://doi.org/10.3168/jds.2010-3722.

Richter, K., M. Haslbeck, and J. Buchner. 2010. The heat shock response: life on the verge of death. *Mol. Cell* 40:253–266. https://doi.org/10.1016/j.molcel.2010.10.006.

Rivera, R. M., K. L. Kelley, G. W. Erdos, and P. J. Hansen. 2004. Reorganization of microfilaments and microtubules by thermal stress in two-cell bovine embryos. *Biol. Reprod.* 70:1852–1862. https://doi.org/10.1095/biolreprod.103.024901.

Romero, R. D., A. Montero Pardo, H. H. Montaldo, A. Rodríguez, and J. Hernández Cerón. 2013. Differences in body temperature, cell viability, and HSP-70 concentrations between Pelibuey and Suffolk sheep under heat stress. *Trop. Anim. Health Prod.* 45:1691–1696. https://doi.org/10.1007/s11250-013-0416-1.

Ronchi, B., G. Stradaioli, A. Verini Supplizi, U. Bernabucci, N. Lacetera, P. A. Accorsi, A. Nardone, and E. Seren. 2001. Influence of heat stress or feed restriction on plasma progesterone, oestradiol-17β, LH, FSH, prolactin and cortisol in Holstein heifers. *Livest. Prod. Sci.* 68:231-241. https://doi.org/10.1016/S0301-6226(00)00232-3.

Rosales-Nieto, C. A., A. Mantey, B. Makela, T. Byrem, R. Ehrhardt, and A. Veiga-Lopez. 2020. Shearing during late pregnancy increases size at birth but does not alter placental endocrine responses in sheep. *Animal* 14:799-806. https://doi.org/10.1017/S1751731119002696.

Rui L. 2014. Energy metabolism in the liver. *Compr. Physiol.* 4:177–197. https://doi.org/10.1002/cphy.c130024.

Rumball, C. W. H., J. E. Harding, M. H. Oliver, and F. H. Bloomfield. 2008. Effects of twin pregnancy and periconceptional undernutrition on maternal metabolism, fetal growth and glucose-insulin axis function in ovine pregnancy. *J Physiol.* 586:1399−1411. https://doi.org/10.1113/jphysiol.2007.144071.

Russel, A. J., J. M. Doney, and R. G. Gunn. 1969. Subjective assessment of body fat in live sheep. *J. Agric. Sci.* 72:451−454. https://doi.org/10.1017/S0021859600024874.

Rutter, W., T. Laird, and P. Broadbent. 1972. A note on the effects of clipping pregnant ewes at housing. *Anim. Prod.* 14:127−130. https://doi.org/10.1017/S0003356100000374.

Rutter, W., T. R. Laird, and P. J. Broadbent. 1971. The effect of clipping pregnant ewes at housing and of feeding different basal roughages. *Anim. Prod.* 13:329–336. https://doi.org/10.1017/S0003356100029779.

Salama, A. A. K., G. Caja, S. Hamzaoui, B. Badaoui, A. Castro-Costa, D. A. E. Façanha, M. M. Guilhermino, and R. Bozzi. 2014. Different levels of response to heat stress in dairy goats. *Small Rumin. Res.* 121:73–79. https://doi.org/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. Pages 11–36 in Animal Welfare in Extensive Production Systems. J. J. Villalba and X. Manteca, ed. 5M Publishing, Sheffield, UK.

Salama, A. A. K., M. Duque, L. Wang, K. Shahzad, M. Olivera, and J. J. Loor. 2019. Enhanced supply of methionine or arginine alters mechanistic target of rapamycin signaling proteins, messenger RNA, and microRNA abundance in heat-stressed bovine mammary epithelial cells in vitro. *J. Dairy Sci.* 102:2469–2480. https://doi.org/10.3168/jds.2018-15219.

Salama, A.A.K., A. Contreras-Jodar, S. Love, N. Mehaba, X. Such, G. Caja. 2020. Milk yield, milk composition, and milk metabolomics of dairy goats intramammary-challenged with lipopolysaccharide under heat stress conditions. Sci. Rep. 10:5055. https://doi.org/10.1038/s41598-020-61900-8.

Sanchez Belda, A. 1994. Historia de la raza ovina Manchega. Pages 11−35 in Ganado ovino: raza Manchega. L .Gallego, A. Torres and G. Caja, ed. Mundi-Prensa, Madrid, ES.

Sanchez-Alavez, M., I. V. Tabarean, O. Osborn, K. Mitsukawa, J. Schaefer, J. Dubins, K. H. Holmberg, I. Klein, J. Klaus, L. F. Gomez, H. Kolb, J. Secrest, J. Jochems, K. Myashiro, P. Buckley, J. R. Hadcock, J. Eberwine, B. Conti, and T. Bartfai. 2010. Insulin causes hyperthermia by direct inhibition of warm-sensitive neurons. *Diabetes* 59:43–50. https://doi.org/10.2337/db09-1128.

Sánchez-Molano, E., V. V. Kapsona, J. J. Ilska, S. Desire, J. Conington, S. Mucha, and G. Banos. 2019. Genetic analysis of novel phenotypes for farm animal resilience to weather variability. *BMC genet.* 20:84. https://doi.org/10.1186/s12863-019-0787-z.

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.* 68:2557–2564. https://doi.org/10.3168/jds.S0022-0302(85)81137-1.

Sano, H., K. Takahashi, K. Ambo, and T. Tsuda. 1983. Turnover and oxidation rates of blood glucose and heat production in sheep exposed to heat. *J. Dairy Sci.* 66:856–861. https://doi.org/10.3168/jds.S0022-0302(83)81867-0.

Sargison, N. D. 2007. Pregnancy toxemia. Pages 359-363 in Diseases of Sheep. 4th ed. I. D. Aitken. Blakwell Publising. Oxford, UK.

Schulze, R. J., M. B. Schott, C. A. Casey, P. L. Tuma, and M. A. McNiven. 2019. The cell biology of the hepatocyte: A membrane trafficking machine. *J. Cell. Biol.* 218:2096–2112. https://doi.org/10.1083/jcb.201903090.

Seal, C., and C. Reynolds 1993. Nutritional implications of gastrointestinal and liver metabolism in ruminants. *Nutr. Res. Rev.* 6:185–208. https://doi.org/10.1079/NRR19930012.

Segnalini, M., U. Bernabucci, A. Vitali, A. Nardone, and N. Lacetera. 2013. Temperature humidity index scenarios in the Mediterranean basin. *Int. J. Biometeorol.* 57:451–458. https://doi.org/10.1007/s00484-012-0571-5.

Sejian, V., M. Bagath, G. Krishnan, V. P. Rashamol, P. Pragna, C. Devaraj, and R. Bhatta. 2019. Genes for resilience to heat stress in small ruminants: A review. *Small Rumin. Res.* 173:42–53. https://doi.org/10.1016/j.smallrumres.2019.02.009.

Sejian, V., R. Bhatta, J. B. Gaughan, F. R. Dunshea, and N. Lacetera. 2018. Review: Adaptation of animals to heat stress. *Animal* 12:s431–s444. https://doi.org/10.1017/S1751731118001945.

Serradilla, J. M., M. J. Carabaño , M. Ramón, A. Molina, C. Diaz, and A. Menéndez-Buxadera. 2017. Characterisation of goats' response to heat stress: tools to improve heat tolerance. Pages 329−347 in Goat Science. S. Kukovics, ed. IntechOpen, Rijeka, HR. https://doi.org/10.5772/intechopen.70080.

Severinsen, M., and B. K. Pedersen. 2020. Muscle-organ crosstalk: the emerging roles of myokines. *Endocr. Rev.* 41:594–609. https://doi.org/10.1210/endrev/bnaa016.

Sevi, A., and M. Caroprese. 2012. Impact of heat stress on milk production, immunity and udder health in sheep: A critical review. *Small Rumin. Res.* 107:1–7. https://doi.org/10.1016/j.smallrumres.2012.07.012.

Sevi, A., G. Annicchiarico, M. Albenzio, L. Taibi, A. Muscio, and S. Dell'Aquila. 2001. Effects of solar radiation and feeding time on behavior, immune response and production of lactating ewes under high ambient temperature. *J. Dairy Sci.* 84:629–640. https://doi.org/10.3168/jds.S0022-0302(01)74518-3.

Shahzad, K., H. Akbar, M. Vailati-Riboni, L. Basiricò, P. Morera, S. L. Rodriguez-Zas, A. Nardone, U. Bernabucci, J. J. Loor. 2015. The effect of calving in the summer on the hepatic transcriptome of Holstein cows during the peripartal period. *J. Dairy Sci.* 98:5401–5413. https://doi.org/10.3168/jds.2015-9409.

Shalova, I. N., J. Y. Lim, M. Chittezhath, A. S. Zinkernagel, F. Beasley, E. Hernández-Jiménez, V. Toledano, C. Cubillos-Zapata, A. Rapisarda, J. Chen, K. Duan, H. Yang, M. Poidinger, G. Melillo, V. Nizet, F. Arnalich, E. López-Collazo, and S. K. Biswas. 2015. Human monocytes undergo functional re-programming during sepsis mediated by

hypoxia-inducible factor-1α. *Immunity* 42:484–498. https://doi.org/10.1016/j.immuni.2015.02.001.

Shyy, T. T., B. B., Asch, and H. L. Asch. 1989. Concurrent collapse of keratin filaments, aggregation of organelles, and inhibition of protein synthesis during the heat shock response in mammary epithelial cells. *J. Cell. Biol.* 108: 997–1008. https://doi.org/10.1083/jcb.108.3.997.

Silanikove, N. 1992. Effects of water scarcity and hot environment on appetite and digestion in ruminants: a review. *Livest. Prod. Sci.* 30:175–194. https://doi.org/10.1016/S0301-6226(06)80009-6.

Silanikove, N. 2000. Effects of heat stress on the welfare of extensively managed domestic ruminants. *Livest. Prod. Sci.* 67:1–18. https://doi.org/10.1016/S0301-6226(00)00162-7.

Silanikove, N., and N. D. Koluman. 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.* 123:27–34. https://doi.org/10.1016/j.smallrumres.2014.11.005.

Silvestri, E., P. de Lange, M. Moreno, A. Lombardi, M. Ragni, A. Feola, L. Schiavo, F. Goglia, and A. Lanni. 2006. Fenofibrate activates the biochemical pathways and the de novo expression of genes related to lipid handling and uncoupling protein-3 functions in liver of normal rats. *Biochim. Biophys. Acta.* 1757:486–495. https://doi.org/10.1016/j.bbabio.2006.02.016.

Smith, W. S, R. Broadbridge, J. M. East, A.G. Lee. 2002. Sarcolipin uncouples hydrolysis of ATP from accumulation of Ca^{2+} by the Ca^{2+} ATPase of skeletal-muscle sarcoplasmic reticulum. *Biochem. J.* 361:277–286. https://doi.org/10.1042/0264-6021:3610277.

Smoliak S., and S. B. Slen. 1972. Wool production of range ewes at three intensities of grazing. *Can. J. Anim. Sci.* 52: 485-490. https://doi.org/10.4141/cjas72-057.

Song, Z., W. Chen, D. Athavale, X. Ge, R. Desert, S. Das, H. Han, and N. Nieto. 2021. Osteopontin takes center stage in chronic liver disease. *Hepatology* 73:1594–1608. https://doi.org/10.1002/hep.31582.

Sonna L. A., J. Fujita, S. L. Gaffin, and C. M. Lilly. 2002. Invited Review: Effects of heat and cold stress on mammalian gene expression. *J. Appl. Physiol.* 92:1725-1742. https://doi.org/10.1152/japplphysiol.01143.2001.

Sphor, L., G. Banchero, G. Correa, M. T. M. Osorio, and G. Quintans. 2011. Early prepartum shearing increases milk production of wool sheep and the weight of the lambs at birth and weaning. *Small Rumin. Res.* 99:44–47. https://doi.org/10.1016/j.smallrumres.2011.03.007.

Spiers, D. E., J. N. Spain, J. D. Sampson, and R. P. Rhoads. 2004. Use of physiological parameters to predict milk yield and feed intake in heat-stressed dairy cows. *J. Therm. Biol.* 29:759–764. https://doi.org/10.1016/j.jtherbio.2004.08.051.

Spiers, D.E. 2012. Physiological basics of temperature regulation in domestic animals. Pages 17−34 in Environmental physiology of livestock. R.J. Collier and J.L. Collier, ed. John Wiley & Sons Inc., West Sussex, UK. https://doi.org/10.1002/9781119949091.ch2.

Srikandakumar, A., and E. H. Johnson. 2004. Effect of heat stress on milk production, rectal temperature, respiratory rate and blood chemistry in Holstein, Jersey and Australian Milking Zebu cows. *Trop. Anim. Health. Prod.* 36:685–692. https://doi.org/10.1023/b:trop.0000042868.76914.a9.

Srivastava A., G. Joshy, and R. K.M. Karuturi. 2019.Transcriptome Analysis. Pages 792−805 in Encyclopedia of bioinformatics and computational biology. S. Ranganathan, M. Gribskov, K. Nakai, C. Schönbach, Academic Press, Selangor, MY. https://doi.org/10.1016/B978-0-12-809633-8.20161-1.

Stephenson, R. G., R. D. Hooley, J. K. Findlay, and P. S. Hopkins. 1980. Effects of heat stress on the lactation performance of ewes accustomed to tropical conditions and the total fluid intake of their lambs. *Aust. J. Biol. Sci.* 33:449–456. https://doi.org/10.1071/bi9800449.

St-Pierre N.R., B. Cobanov, and G. Schnitkey. 2003. Economic losses from heat stress by US livestock industries. *J. Dairy. Sci.* 86:E52-E77. https://doi.org/10.3168/jds.S0022-0302(03)74040-5.

Streffer C. 1982. Aspects of biochemical effects by hyperthermia. *Natl. Cancer Inst. Monogr.* 61:11–17.

Symonds, M. E., M. J. Bryant, and M. A. Lomax. 1986. The effect of shearing on the energy metabolism of the pregnant ewe. *Br. J. Nutr.* 56:635–643. https://doi.org/10.1079/BJN19860144.

Symonds, M. E., M. J. Bryant, and M. A. Lomax. 1989. Lipid metabolism in shorn and unshorn pregnant sheep. *Br. J. Nutr.* 62:35–49. https://doi.org/10.1079/bjn19890006.

Tanaka, M., Y. Kamiya, M. Kamiya, and Y. Nakai. 2007. Effect of high environmental temperatures on ascorbic acid, sulfhydryl residue and oxidized lipid concentrations in plasma of dairy cows. *Anim. Sci. J.* 78: 301–306. https://doi.org/10.1111/j.1740-0929.2007.00439.x.

Tao, S., R. M. Orellana, X. Weng, T. N. Marins, G. E. Dahl, and J. K. Bernard. 2018. Symposium review: The influences of heat stress on bovine mammary gland function. *J. Dairy Sci.* 101:5642–5654. https://doi.org/10.3168/jds.2017-13727.

Thom, EC. 1959. The discomfort index. *Weatherwise* 12:57–59. https://doi.org/10.1080/00431672.1959.9926960.

Toivola, D. M., P. Strnad, A. Habtezion, and M. B. Omary. 2010. Intermediate filaments take the heat as stress proteins. *Trends Cell Biol.* 20:79–91. https://doi.org/10.1016/j.tcb.2009.11.004.

Valko, M., C. J. Rhodes, J. Moncol, M. Izakovic, and M. Mazur. 2006. Free radicals, metals and antioxidants in oxidative stress-induced cancer. *Chem. Biol. Interact.* 160:1–40. https://doi.org/10.1016/j.cbi.2005.12.009.

Volloch, V., and S. Rits. 1999. A natural extracellular factor that induces Hsp72, inhibits apoptosis, and restores stress resistance in aged human cells. *Exp. Cell Res.* 253: 483–492. https://doi.org/10.1006/excr.1999.4682.

Walter, P., and D. Ron. 2011. The unfolded protein response: from stress pathway to homeostatic regulation. *Science* 334:1081–1086. https://doi.org/10.1126/science.1209038.

Wang, X., Xin, H., Xing, M., Gu, X., and Hao, Y. 2022. Acute endoplasmic reticulum stress induces inflammation reaction, complement system activation, and lipid metabolism disorder of piglet livers: A proteomic approach. *Front. Physiol.* 13:857853. https://doi.org/10.3389/fphys.2022.857853.

Wang, Y., X. Jia, J. Hsieh, M. S. Monson, J. Zhang, D. Shu, Q. Nie, M. E. Persia, M. F. Rothschild, and S. J. Lamont. 2021. Transcriptome response of liver and muscle in heat-stressed laying hens. *Genes (Basel)* 12:255. https://doi.org/10.3390/genes12020255.

Wang, Z., M. Gerstein, and M. Snyder. 2009. RNA-Seq: a revolutionary tool for transcriptomics. *Nature Rev. Genet.* 10:57–63. https://doi.org/10.1038/nrg2484.

Welch, W. J., and J. P. Suhan. 1985. Morphological study of the mammalian stress response: characterization of changes in cytoplasmic organelles, cytoskeleton, and nucleoli, and appearance of intranuclear actin filaments in rat fibroblasts after heat-shock treatment. *J. Cell Biol.* 101:1198–1211. https://doi.org/10.1083/jcb.101.4.1198.

West, J. W. 2003. Effects of heat-stress on production in dairy cattle. *J. Dairy Sci.* 86: 2131–2144. https://doi.org/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.* 93:644–655. https://doi.org/10.3168/jds.2009-2295.

Willis, W. T., M. R. Jackman, M. E. Bizeau, M. J. Pagliassotti, and J. R. Hazel. 2000. Hyperthermia impairs liver mitochondrial function in vitro. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 278:R1240–R1246. https://doi.org/10.1152/ajpregu.2000.278.5.R1240.

Wojtas, K., P. Cwynar, and R. Kołacz. 2014. Effect of thermal stress on physiological and blood parameters in merino sheep. *J. Vet. Res.* 58:283-288. https://doi.org/10.2478/bvip-2014-0043.

Wu, J., W. Zhang, and C. Li. 2020. Recent advances in aenetic and apigenetic modulation of animal exposure to high temperature. *Front. Genet.* 11:653. https://doi.org/10.3389/fgene.2020.00653.

Yousef, M. K. 1985. Stress physiology in livestock. CRC Press, BocaRaton, FL.

Yousef, M. K., H. D. Johnson. 1966. Calorigenesis of cattle as influenced by growth hormone and environmental temperature. *J. Anim. Sci.* 26:1087–1093. https://doi.org/10.2527/jas1966.2541076x.

Yue, S., Z. Wang, L. Wang, Q. Peng, and B. Xue. 2020. Transcriptome functional analysis of mammary gland of cows in heat stress and thermoneutral condition. *Animals (Basel)* 10:1015. https://doi.org/10.3390/ani10061015.

Zhao, Q. L., Y. Fujiwara, and T. Kondo. 2006. Mechanism of cell death induction by nitroxide and hyperthermia. *Free Radic. Biol. Med.* 40:1131–1143. https://doi.org/10.1016/j.freeradbiomed.2005.10.064.