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Quantifying the response to heat stress in Sarda dairy sheep housed indoor with indicators measurable on field

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DEDICATION

I declare that this thesis and the results contained therein are my original thoughts and have never been submitted to another institution for consideration for a PhD or a degree of a similar nature.

Fabio Fulghesu

GENERAL SUMMARY

Sheep are considered one of the most favoured species to adapt to climate change. Nevertheless, they suffer heat stress more intensively than cattle due to their high metabolic effort per kg of BW and the wool coat. Biological markers of heat stress need to be defined at field level. This PhD dissertation was developed to quantify the response to heat stress of Sarda sheep housed indoor in several experiments. The Thesis was developed within 7 Chapters.

Chapter 1 presents a literature review on the heat stress in sheep and, at its end, the main objectives of the work.

In Chapter 2, was presented a trial in which fifteen Sarda dairy ewes were divided in three groups different for milk yield (MY): High MY, Low MY and Dry with 1900, 1200, 0 g/d of milk per ewe (n=5), respectively. Air temperature (AT) and relative humidity (RH) were recorded and nine physiological variables were measured on the individual animals, five times per day, for 60 days. With increasing of THI classes, a significant increase was observed for all animal measures ($P< .001$). Lactating animals showed high stress in comparison to dry groups. In conclusion, respiration rate (RR) was the most valuable marker for heat stress in housed sheep.

In Chapter 3, records from dry ewes raised in the same experimental barn and in two different seasons (winter 2022 and summer 2021), were studied to quantify the ranges of RR and rectal temperatures. RR and rectal temperature (RT) were not significantly affected by AT in winter, showing low values and small standard deviations, equal to 27.8±7.2 breath/min and 38.86±0.55 °C, respectively. In summer, RR and RT were equal to 71.1±34.8 breath/min and 38.95±0.30, respectively. Daily variation of indicators showed that ewes have strong summer accumulation of heat from morning to evening even with medium THI values.

In Chapter 4, twenty-one Sarda dairy ewes, separated in 3 groups fed different diets for the NDF quality, were monitored for 21 days in summer 2022. RR, RT, DMI and MY were recorded. DMI was not affected by THI but MY showed a strong decrease with THI>76 (-11.2% compared to THI=71-72; $p<0.05$) without differences among groups. RR ranged from 30-50 breaths/min (at THI of 65) to a max of 90-170 breaths/min (at THI of 80) as predictable by the equation: $RR = 0.2555^{\wedge 0.0739*THI}$.

In Chapter 5, was presented a trial carried out on 16 sheep housed in ventilated hoods for direct measurements of methane emissions. The data showed that respiration rate is positively correlated with methane emissions during measuring trials.

In Chapter 6, a descriptive statistical analysis was carried out using biometrics measurements of ewes to develop a simple geometric model to estimate volume and surface of the animals. Furthermore, a conceptual model of heat production and dissipation in sheep was developed as preliminary basis to develop a dynamic model of heat stress response in sheep.

In Chapter 7, the general conclusions and practical implications of the work were presented for scientific and applicative uses.

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LIST OF ABBREVIATIONS

ADF	Acid detergent fiber
ADL	Acid detergent lignin
AHQ	Dehydrated chopped alfalfa hay (high quality)
ALQ	Dehydrated chopped alfalfa hay (low quality)
AT	Air temperature
BCS	Body condition score
BF	Bioflavonoids
BW	Body weight
CLD	Causal loop diagram
CNT	Control group
CP	Crude protein
CT	Tannins
DM	Dry matter
DMI	Dry matter intake
DOM	Digestible organic matter
EE	Extract ether
EO	Essential oils
ET	Ear temperature
HLI	Heat load index
HR	Pulse heart rate
ID	Identification number of individual animals
ME	Metabolizable energy
MY	Milk yield
NDF	Neutral detergent fiber

OH	Dehydrated chopped oat hay
OM	Organic matter
OX	Blood oxygen saturation
PT	Ocular globe temperature
RH	Relative humidity
RR	Respiratory rate
RT	Rectal temperature
SA	Surface area
SA	System analysis
SD	System dynamic
SE	System engineering
ST	Skin temperature
THI	Temperature humidity index
TMR	Total mixed ration
TRT	Experimental treatment
UT	Udder temperature
VT	Vagina temperature

CHAPTER 1

Introduction and objectives of the thesis

1. INTRODUCTION

1.1. Climate and heat stress problems in Small ruminants

Climate change is one of the major problems of our century. According to reports from the United Nations Intergovernmental Commission on Climate Change (IPCC), the global surface temperature of the planet has increased by 0.74 ± 0.18°C during the last 100 years up to 2005 and according to their forecasts, it could further increase by about 1.1-6.4°C over the course of the 21st century. This progressive increase in temperatures has a strong impact on the entire zootechnical sector, causing important production and reproductive losses, especially in more intensive systems. Climate change causes problems regarding feed and water resources, also to the detriment of the species and diversity of fodder crops in pastures. This creates inconvenience for the breeding of sheep and goats which in various areas of the world occurs through grazing. Changes in food and water resources therefore cause stress (Altınçekiç and Koyuncu, 2012). With temperatures. In high environmental conditions, together with relative humidity (RH), air flow and solar radiation, body temperature increases above critical levels creating physiological side effects in animal production (Kadim et al. 2008). In 2021, the global average annual temperature anomaly was 1.4 ºC and ranks, together with 2019, as the third warmest year between 1961 and 2021 (2016 in second position with 1.6 ºC; 2020 in first position with 1.7°C). As regards the period between 2012 and 2021, this was on average 1.3°C warmer than the period between 1951 and 1980 (warming of 0.3°C in the decade 2002-2011; of 0.7°C in decade 1992-2001; FAO, 2022; Tüfekci, 2023).The meteorological variables strongly condition heat stress (first and foremost temperature and relative humidity) and just like man, animals tolerate dry heat conditions better than humid heat conditions, so when high temperatures are accompanied high relative humidity values, create a microclimate that is disadvantageous for the well-being and performance of the farmed species. Taking into account the fact that animals have a good capacity for heat exchange with the environment, which depends on the direct radiation of the sun, on the radiation reflected by the clouds and the ground and on the radiation scattered by the particles in the air, the variable that most influences negatively the evaporative mechanisms is therefore the relative humidity that hinders perspiration and therefore the dispersion of heat. The other factors that influence heat exchanges are wind speed and rainfall. The movement of the air, in fact, increases the thermal exchanges because, penetrating inside the hair, it eliminates the warm air cushion guaranteed by the wool and fur; therefore it has positive effects in summer and negative effects in winter. Precipitation, on the other hand, assisted by high humidity, wets the hair which thus loses its ability to thermally insulate the animals. McDowell (1972) has indicated, as optimal environmental conditions for most livestock species, a temperature of 13-18°C, a relative humidity of 60-70%, a wind intensity of 3-5 km/h and an average level of solar radiation. In response to the need to find a system for evaluating effective heat stress (easy and reliable to use), starting from the twentieth century the THI (Temperature-Humidity Index) was developed, a parameter which takes into account only the temperature parameters relative humidity of the environment. In order to be able to fully evaluate the level of stress of the animals, it is advisable to take into account not only the environmental factors but also the physiological factors. Animals exposed to high ambient temperatures respond with an increase in the dissipation of excess body heat, so as to balance the excessive thermal load, therefore through the evaporation of water through the respiratory tract first and through sweating after.

Whereas animal raised outdoor requires shade and water availability, when reared indoor, dairy sheep require a proper ventilation and facilities management for temperature control. The cardinal weather variables demonstrated to impose significant effects on the livestock production are temperature, humidity, wind velocity, direct and indirect solar radiation and photoperiod, and among this ambient temperature is proved to be most crucial (Rashwan Ali et al. 2004). Direct impacts of climate change may result in higher surface temperature, atmospheric CO_2 levels and modified precipitation pattern, and all these consequences eventually lead to impaired crop and livestock productivity (Hatfield et al. 2008; Sejian et al. 2012). In the changing climate scenario, the fate of sheep husbandry in many parts of the world raises serious questions. It is expected that climate change may cause shifting of sheep from one region to another, change in breed composition, change in livelihood and nutritional security of farmers, shifting trend of sheep breeds from wool to mutton type, emergence, re-emergence of newer diseases, etc. Climate change impairs the livestock production potential directly by altering temperature, humidity, solar radiation and wind velocity and indirectly by influencing the feed digestibility, feed availability and quality, pest and disease incidences, etc. Fundamentally, climate change disturbs the sheep production efficiency in several ways including (a) deleterious effects on forage availability and quality, (b) modified pest and disease distribution and (c) direct impacts of fluctuating weather on animal production performances (Smith et al. 1996). Table 1 illustrates the effects of environmental stresses on sheep production. To proper manage the flocks under several conditions of temperature and microclimate constraints is necessary to better understand the relationship among environment and animal physiological adaptive response to heat dissipation, and to quantify the effect of temperature increase and microclimate on production losses.

Table 1. Sheep production and climate change (adapted from Sejian et al., 2017).

Growth	Meat production	Milk production	Reproduction	Availability of feed and water	Distribution of livestock diseases
Reduced average daily gain	Transport-related mortality	Reduced milk yield	Reduced intensity and duration of oestrus	Reduced pasture availability	Altered patterns diseases in animals
Reduced body weight	Reduction in meat quality	Poor milk quality	Increased embryo mortality	Reduced quantity of feed and fodder	Emergence of new diseases
Reduced BCS	Decrease in protein and marbling	Milk delivery oscillation due to heat waves	Reduced fertility	Reduced micronutrients content in feed	Variations in the predominance of existing diseases
Reduced birth weight	High cooking losses in meat	Increased seasonality	Low progesterone Decreased quality of oocytes Reduced LH Reduced oestradiol	Reduced water quantity and quality available for livestock	Variations in distribution and the abundance of disease vectors

1.2. Relationship between environment and heat stress

Temperature humidity index or THI

A parameter recurrently mentioned in the previous part was THI, but let's see the meaning. THI born to a twentieth century necessity to develop a method to quantify initially the level of human discomfort during summer, after also to quantify animals heat stress. THI is the translation in numerical terms of the environmental conditions that considers temperature and relative humidity about a particular place, beginning a forecast system to assess the possible threat or danger to the animals due to climatic variations. It is usually expressed in °F and presented like a score from 50 to 100. However, in generally, it has two leaks as it does not take into account solar radiation and wind speed, which are also considered important weather parameters greatly influencing the animal performances (Sejian et al., 2018). Over the years various equations have been created to quantify THI (°F): Kliber's equation (1964) $THI = (1.8 X T – ((1 - RH/100) (T – 14.3)) + 32$; Kelly and Bond (1971) equation $THI = (1.8 x T + 32 - (0.55 – 0.0055 x RH) x (1.8 x T – 26)$ (Wildridge et al., 2018) o $THI = AT – 0.55 x (1 – RH) x (AT – 58)$ (Sejian et al., 2017) o $THI = \{T - [0.55 \times (1 - RH)] \times (T - 14.4)\}$

(Finocchiaro et al., 2005). Those equations consider air temperature and air relative humidity, whereas more recently, Finocchiaro at al (2005) used THI = Tbd °C – [0.55 x (1 – RH)] x (Tbd °C – 14.4), that consider dry bulb temperature and relative humidity. It's been observed, as reported in following paragraphs, that in relation to a particular combination of air temperature and relative humidity, there are physiological and metabolic changing in animals, with performances decreases to death risk: increase of respiratory rate, rectal temperature, pulse rate, skin temperature, sweating, decrease of milk production, reproductive capacity, and others.

In addition to Temperature Humidity index a more recent indicator has been developed, named the Heat load index (HLI). It overcomes the perceived deficiencies in the THI index using a combination of black globe temperature, air movement and relative humidity. The HLI uses two equations based on the threshold value of black globe thermometer (Gaughan et al., 2008):

$$HLI_{BG\geq 25} = 8.62 + (0.38 * RH) + (1.55 * BG) - (0.5 * WS) + e^{(2.4-WS)}$$

$$HLI_{BG<25} = 10.66 + (0.28 * RH) + (1.3 * BG) - WS$$

where BGT is the black globe temperature in °C, RH the relative humidity in %, WS the wind speed in m/s and e the exponential. The HLI is an ideal indicator of the temperature status of the animal, and it can explain 93% of the alterations in panting score on beef cattle (Gaughan et al., 2008).

1.3. Relationship between environment and animal

Thermoregulation and homeostatis

Hill et al. (2012), define thermoregulation as a fundamental physiological process with implications not only for the organismal level but also from an ecological standpoint;

they define this process as a species thermal niche. Thermoregulation is influenced by: species and breed, characterized by different size (due to the principle according to which large animals are more resistant to cold and small animals are more resistant to heat), thickness of wool, deposit of subcutaneous fat and sweat glands numbers; age (young animals are more resistant to heat than adults); physiological stage (lactating animals are less resistant to heat than dry animals, due to greater ingestion of energy and the consequent production of endogenous heat).

Homeostasis is the living organism attitude to preserve their characteristics from variation of environment conditions through chemical-physical, biological and behavioural self-regulation mechanisms. A subject in normal conditions produces energy and dissipates it, in analogy with what the first law of thermodynamics on energy conservation says, which implies that if during the day changes in temperature occur, something must intervene that allows for keep it constant. Heat is a form of energy that passes from one system to another until temperature differences stop and thermal equilibrium is reached. When body temperature exceeds environmental temperature, sensible heat must flow through the body tissue and the fur of the animal to the environment. When in a thermo-neutral environment this flux exceeds the rate at which heat is produced by metabolism, evaporative heat loss is minimal and any decrease of environmental temperature must be balanced by an increase in metabolic rate (Boldrini et al., 2018; Figure 1). Temperature regulation in mammals is highly organized to respond to several factors that can generate thermal changes: environment, reproductive phases, nutritional and inflammatory processes that affect them. The skin is an important organ that is involved in mechanisms related to thermal homeostasis in responses to thermal imputs. This is because it is at the forefront of detecting thermal variations. Heat dissipation through the blood flow occurs through vasomotor processes:

vasodilation and vasoconstriction (Mota-Rojas et al., 2021). When environmental temperatures are high, skin vasodilation occurs. Its objective is to divert the central and deep circulation towards peripheral circulation in order to be able to dissipate heat using the blood as a vehicle. Two mechanisms of vasodilation are known: active vasodilation (increasing blood flow by increasing nerve activity) and passive vasodilation (increasing blood flow but reducing vasoconstrictor nerve activity) (Smith and Johnson, 2016; Wu et al., 2017). Under conditions of cold stress, the sympathetic nerve fibers that innervate the cutaneous vascular system are activated, causing cutaneous vasoconstriction capable of reducing heat transfer with the environment and capable of conserving heat in the body core of the organism (Madden and Morrison, 2019). Thermal regulation is influenced, among other factors, by the characteristics of the wool. Sheep wool is related to breed, age, sex and environmental conditions, such as temperature, relative humidity and wind. It is also an insulating layer that protects the animal from both heat and cold. The type of wool is extremely important. Open wool of the dairy breeds provides less protection against solar radiation than the closed wool of the Merino (Pennisi et al., 2004). Heat exchange between animal and environment is an important component of metabolic heat production, in addition to growth, milk production, pregnancy and activity components. Animal's body surface is the interface for convective, radiant and evaporative heat loss from the skin, supplemented by convective and evaporative heat loss through the respiratory system. Heat is released from the animal by evaporative heat loss and non-evaporative heat loss. Evaporative heat, from the respiratory tract and skin, is relative (but not linearly proportional) to the heat stored in the body. Conversely, non-evaporative heat loss is determined by the animal-ambient temperature gradient and by the surface of the animal. In fact, the characteristics of the environment play an important role, such as dry bulb temperature, humidity, air movement (Gagge and Gonzalez, 2010) as well as the physical properties of the skin surface: its temperature, humidity due to sweating, the presence of wool.

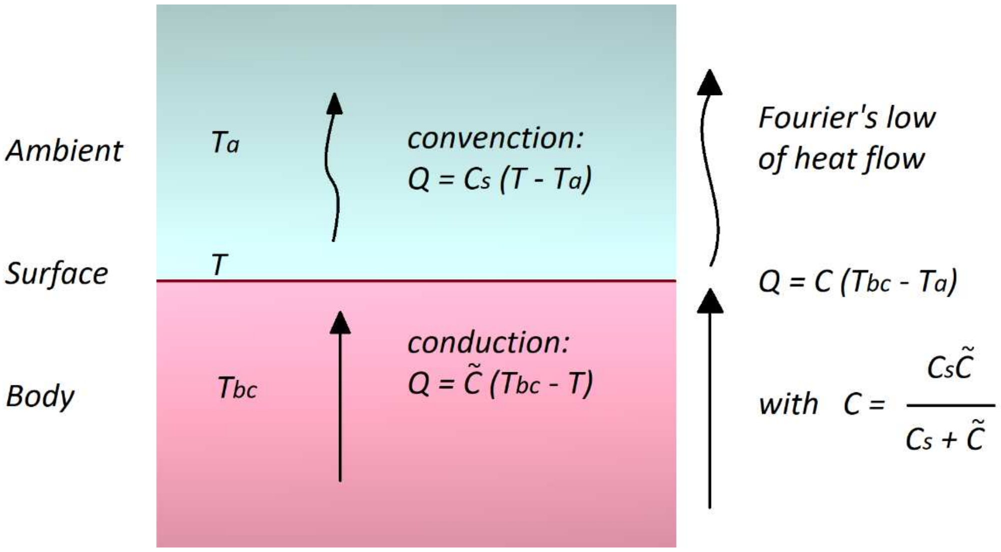

Figure 1. Diagram of heat flow from core of animal's body, through internal tissues and fur, to the environment. Tbc = Nucleus of the animal's body; T = animal temperature; Ta = Ambient temperature. This system is described by the equation Q = Cs (T − Ta) (where: T = surface temperature; Cs = surface convection coefficient; Boldrini et al., 2018).

Thermoneutral heat production

The thermoneutral heat production is both dependent and is a consequence of the animal’s metabolizable energy intake. Its inefficiencies are associated to the metabolic processes taking place, independently, by thermal environment direct effect. It’s important to realize that total heat production may be greater, but never less, than the thermoneutral heat production. On the contrary, sensible animal heat demand is determined by thermal environment and by thermal characteristics of the animal, independently by metabolizable energy intake (Bruce, 1993).

Thermoneutral zone

Environment influence life on livestock, principally with air temperature, conditioned in turn by wind, precipitations, humidity and solar radiation. All of these meteorological characteristics are managed by animals through behaviour, physiological and metabolic changes that alter the partition of dietary energy.

Sheep and goats are homeothermic animals that maintain relatively constant body temperature balancing heat produced from metabolism and heat gained or lost from environment. There are two situations that cannot be tolerated for a long time: the first is a cool environment that conduce to hypothermia condition; second is a hot environment that on the contrary conduce to hyperthermia (Figure 2). Usually, an animal lost continually sensible (conduction, convention, radiation) and insensible heat (evaporation). What regulates the heat exchanges between animals and environment are same environment thermal demand (cooling power) and the animal resistance to heat flow of the tissue, skin and its cover. Animals takes heat from environment when its temperature exceeds body core temperature and automatically animals spent energy to expel it. The animal’s problem in contrasting a hot environment is the reduced thermal gradient between body core and the environment, and so the reduced loss capacity for sensible heat (National Research Council, 1981).

Animals gains environmental heat when surrounding temperature or air temperature is higher than animals skin temperature; on the other hand, the sweating and panting mechanisms are limited by air vapor pressure but enhanced by air movement. It is possible to identify an optimal condition in which animals conducted undisturbed their activities. This is told thermoneutral zone, characterized by a range of temperatures variable according to the various species. Generally, the thermoneutral zone promotes the maximum performances and least stress, without appearance of feed intake reductions, increase of respiratory rate, rectal temperature and sweating, becoming the best condiction of animal welfare. Considering the schematic relationship of animal's body core temperature, heat production and environmental temperature adapted from Curtis (1981), thermoneutral zone is delimited by lower critical temperature, beyond which there is cooling stress up to hypothermia and death (extra heat is produced to meet the environmental energy demand); and by upper critical temperature, beyond which there is heat stress and death (reduced heat production with decrease of energy intake) (Figure 2). The net result in both situations, is an altered energetic efficiency, which can require dietary changes in nutrient-to-energy ratios. In summary, inside thermoneutral zone, total heat production is approximately constant.

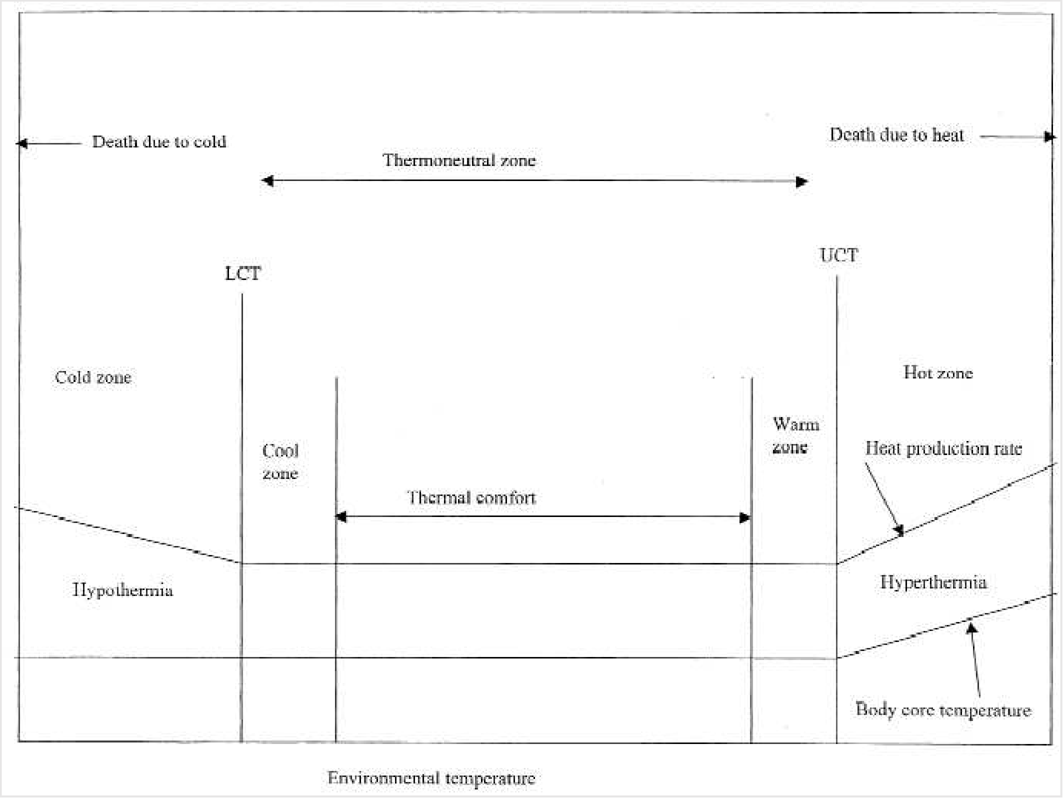

Figure 2. Thermoneutral and stress zones of animal's body temperature; heat production rate vs. environmental temperature (adapted from Curtis, 1981). LCT=lower critical temperature; UCT=upper critical temperature (adapted from Kadzere et al., 2002).

1.4. Energy balance

With the increase of temperatures there is an increase in nutritional, water and salt requirements to allow the animal to release heat through sweating and breathing. On the contrary, the ingestion, instead of increasing in a directly proportional manner, undergoes a reduction to limit the production of metabolic heat. Once an animal starts the thermoregulation mechanisms, like the increase in the respiration rate, the energy is diverted from the needs of growth and production towards the maintenance of homeostasis. This roughly translates into an increase in energy requirements of 7-25%, due to the start of the thermoregulation mechanisms (Sevi and Caroprese, 2012). Therefore, the animal's energy efficiency is significantly compromised during heat stress.

Heat stress causes in animals changes like the decrease of feed intake and its efficiency of utilization, disturbances in water, protein, energy and mineral balances, enzymatic reactions, hormonal secretions and blood metabolites (Marai et al., 2007), reduction of fecal and urinary water losses, increase in sweating, respiration and heart rates (Silanikove, 2000). Dealing about the metabolizable energy (ME) intake in the food we can sustain that it introduces energy into the metabolic system. The animal responds by using its metabolism to distribute the metabolizable energy in ways which maintain an energy balance and presumably satisfy its goals according to their biological importance at any given time. The energy balance can be expressed as an equation:

F (metabolizable energy intake) = C (energy to the conceptus) + B (energy to body tissue) + M (energy to milk) + Q (sum of sensible and latent heat flows to the environment) (Bruce, 1993).

The decrease of energy intake along with elevated energy expenditure for maintenance and with additional energy requirements for growth, gestation and lactation determine a decrease of energy balance that consequently could end in a negative energy balance (Slimen et al., 2019).

The target of feed intake decrease in heat environment, is to create less metabolic heat in that feeding increment produce a big quantitative of heat. Heat stress increase by 30% the maintenance requirements and the low energy intake would not be enough to cover daily requirements, which results in a bod weigh loss (Hamzaoui et al., 2013).

Therefore, the loss of body weight is associated with an increasing of energy expended for heat dissipation and with a decreasing of the energy to be allocated to production factors. The heat stressed animal enter in a bioenergetic state similar to the negative energy balance observed in early lactation (but not to the same extent) (Sarangi, 2018). In essence the negative energy balance is associated with changes in the metabolism and in the hormonal system. One of the most important indicators of defensive dissipation mechanism of animals, particularly important in sheep (Brockway et al., 1965), is the respiratory rate in order to facilitate the dissipation of heat excess.

1.5. Metabolic Indicators of Heat stress

The most common metabolic indicators that can be directly observed at farm level are following listed and described:

Respiration rate

The target of respiration is the elimination of carbon dioxide (CO_2) from tissues of the body and the assumption of oxygen (O_2) under thermo-normal conditions. The evaporation of moisture from respiratory tract is a strategy also used for maintaining thermal balance in animals. It's been observed that under neutral environmental temperature (12 °C) sheep lose approximately 20% of total body heat via respiratory tracts (Marai et al., 2007; Figure 3). Thus the respiratory rate in cold stress is reduced to prevent hypothermia. Otherwise, the respiration rate can be an early warming signal of heat stress conditions in livestock (Rashamol et al., 2018). When the environmental temperature increase over the limit, respiratory evaporative cooling mechanism fails. In fact, when environment temperatures and humidity increase, a protection mechanism that increases the rhythm of respiratory rate is activated. Normally respiration rate increases during summer with heat stress conditions (Hyperthermia) and on the contrary decreases during winter with cool stress conditions (Hypothermia). At high air temperature (35 °C) the moisture loss increases for approximately 60% in sheep (Thompson et al., 1985) and, in sheep as equal in goats, the body temperature can increase to a point at which well-being and productive performances are compromised. The principally reason of compromission of productive performances is the reduction of feed intake to decrease the metabolism heat production, that consequentially reduce the transport of nutrients for the synthesis of milk. Respiration rates for adult goats ranges between 15-30 breaths/min and for adult sheep are 20-30 breaths/min (Jackson and Cockcroft, 2002). An important data comes from the study of Hamzaoui et al. (2013), he observes that the respiration rates increase in thermo-neutral goats from 8:00 to 17:00 h in accordance with the increment of environment temperature from 15 to 20°C. Furthermore, in heat stressed goats at 8:00 h exposed to 30,5°C during the night (THI 77) compared with thermo-neutral goats al 17:00 (THI 65) he observed that the respiration rates were greater in heat stressed goats (+13 breaths/min) through the day, but to a lower extent during the night. Marai et al. (2007) observes in sheep that respiratory rates during summer increase through the day. In fact, it was lower during early morning time (8:00h) than the hot time of the day (15:00h). A study of Phulia et al. (2010) reported that respiration rate increase from 43.66 breaths/min at 38.97 °C to 77.33 breaths/min at 39.35 °C when goats were kept for 6 h in heat stress conditions during summer. Therefore, in goats and in sheep it's been observed an adaptation of animals to heat stress conditions during time. In the first case Hamzaoui et al. (2013) note that respiration rate and rectal temperature values, in heat stressed animals, packed during the first week of experiment and then gradually decreased; in second case, McManus et al. (2009a) analysing the relationship among coat and physiological traits in five sheep groups, found that white coat colour and shorter hair were related to lower rectal temperature, respiratory rate and heart rate, which suggest better adaptation to heat stress. This suggest that breed and physiological characteristics are very important to understand the differences between various levels of adaptability between animals. Vieira at al. (2023) observed that, under heat stress conditions, Suffolk and Dorper breeds presented alterations in respiration rate before when the temperature and humidity index increased, contary to results of Hampshire Down breed on the same variable that was not affected by critical wheater conditions; furthermore Morada Nova breed that between hair breeds was the most resistant in respiratory rate and rectal temperature. The respiration rate of sheep is the major way of thermal dispersion in conditions of high environmental temperatures (Piccione et al., 2008) and it represents a significant and accessible indicator in evaluating stress (Pennisi et al., 2004).

Rectal temperature

Rectal temperature, together with respiratory rate, is a good index of heat stress condition of animals and it is considered as an important measure of physiological status (Rashamol et al., 2018). With rectal temperature we understand the measure of animal's core body temperature and his increasement represent the failure of thermoregulatory mechanism (with respiration and sweat). So, it is an indicator of heat balance and can be used to assess the adversity of heat stress, which can affect growth, lactation and reproduction (West, 1999). It's influenced by metabolic processes and radiation heat and consist to a natural passageway for dissipation of extra heat to maintain physiological homeostasis and normal body temperature. Normal rectal temperatures for goats ranges between 38.3-40.0 °C; for sheep 38.3-39.9 °C. Whit an increase of the air temperature from 18 °C to 35 °C as consequence the rectal temperature increase in sheep (Marai et al., 2007). With a rectal temperature of 42 °C the animal risks his life (Thwaites, 1985). Also in this case a negative trend of this parameter is registered during summer. Comparing rectal temperatures in sheep, at 8:00 h they are markedly lower than at 12:00 and 16:00 h, being in the latter two moments are very similar (Shalaby, 1985). In goats has been observed that also the rectal temperature (as respiratory rate in the other paragraph) increases in thermo-neutral animals from 8:00 to 17:00h when air temperature increases from 15 to 20°C

(Hamzaoui et al., 2013). Rectal temperature starts to rise above normal conditions, at a temperature of 32 °C; open mouth panting begins at a rectal temperature of 40 °C and if the RH is below 65% (Srikandakumar et al., 2003). This parameter is also influenced by breed, in fact Bergamasca and Poll Dorset × Dorper in Vieira et al. (2023) study, demonstrated a strond alteration in rectal temperature contrary to Morada Nova breed. Rectal temperature is a good indicator of thermal balance and may be used to assess the adversity of the thermal environment affecting the growth, lactation and reproduction of dairy ewes. His variations are a sensitive indicator of physiological ewes' response to heat stress, that it is nearly constant under normal conditions (Piccione et al., 2008).

Pulse rate

Pulse rate is a physiological parameter, reliable indicator of heat stress problem in livestock (Das et al., 2011). Cardiorespiratory system is influenced by season, day timings, ambient temperature, humidity and exercise (Marai et al., 2007). Normal range resting pulse rate (beats/min) is 70-90 in sheep and 70-100 in goats (Jackson et Cockcroft, 2002), but also in this case high temperatures influence them. The rate undergoes an increase in case of exposure to high ambient temperatures (Aboul-Naga, 1987) causing an acceleration of the blood flow in such a way as to make a greater dispersion of heat possible. With an increase of pulse rate, the body loses sensible heat (conduction, convection, radiation) and latent heat (water diffusion from the skin) by increasing blood flow from the core to the surface (Gupta et al., 2013; Hooda and Upadhyay, 2014). It reflects the homeostasis of circulation along with the general metabolic status (Marai et al., 2007). It's been reported that heart rate increase during hottest hour of the day (15:00) in sheep that have libitum access to water due to increase of cutaneous blood flow (Alexiev et al., 2004) and also hepatic blood flow tend to be greater at 35 °C than 20 °C. A decrease of heart rate is related to a decrease of metabolic rate and the vasodilation stimulates the pilomotor centre to flatten the hair cover to allow better heat dissipation through conduction, convection and radiation (Marai et al., 2007). A fast pulse rate can be attributed to two causes: i) the increase of the muscular activity controlling the rate respiration and ii) the reduction in resistance of peripheral vascular beds and arteriovenous anastomoses (Gupta et al., 2013).

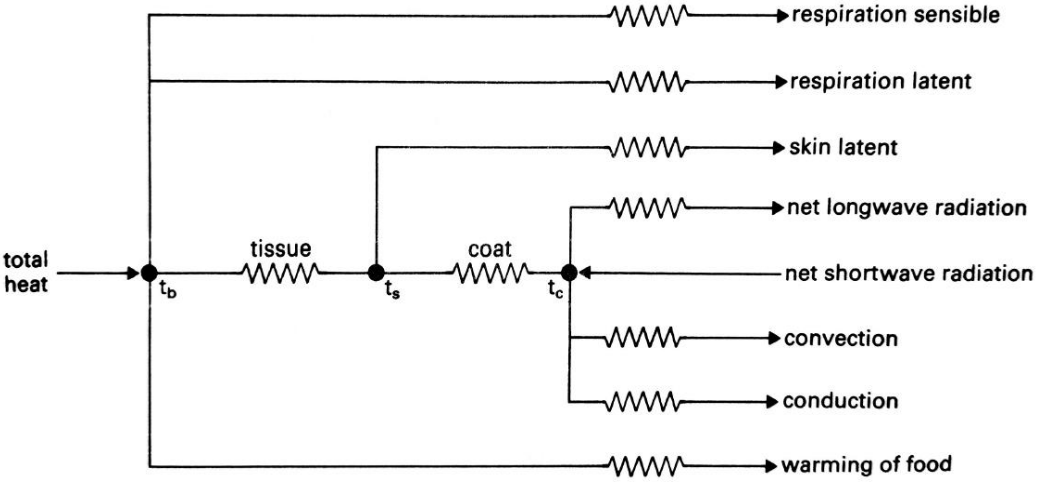

Figure 3. A scheme of the animal-environment energy flows (Bruce, 1993).

Skin temperature

Skin is the most important apparatus for heat exchange between the body and the surrounding environment to balance excessive heat load (Figure 4). Skin temperature depend to the level exposition to sun rays of the different body part and to the skin of blood flow. This parameter was higher during summer season in sheep (Fahmy, 1994) in respect to winter and, similarly to rectal temperature in sheep was lower at 8:00 than 12:00 and 16:00 (being similar in the latter two) (Shalaby, 1985). Generically, an increase of ambient temperature influences the skin temperature as the time of the day and the season.

But when an ambient temperature is greater than skin temperature, the temperature gradient between the body surface and the environment decreases, impeding heat dissipation in this case by an evaporative mechanism (Ribeiro et al., 2018). When the environmental temperature rises to 36 °C, ears and limbs of sheep dissipate a high quantity of heat, because of this area contributo about 23% of the body surface area (Johnson, 1987). Okoruwa (2014) observed an increase in skin temperature of goats exposed to 40.68 °C. It was attributed to the heat stress, which caused vasodilatation of skin capillary bed and consequently increased the blood flow to the skin surface to facilitate heat dissipation (McManus et al., 2009b). Also in goats skin temperature could be directly related to ambient solar radiation levels (Schutz et al., 2011).

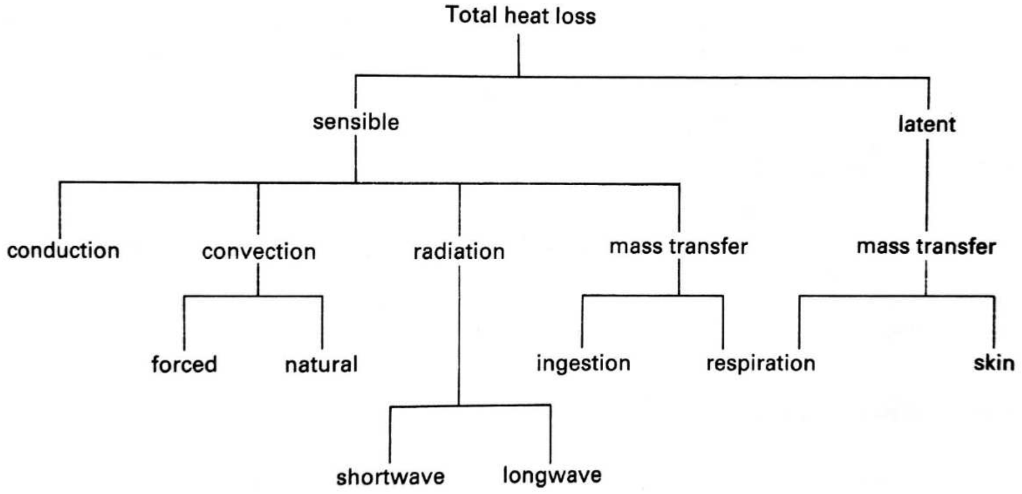

Figure 4. Scheme of the components of total heat loss (Bruce, 1993).

Sweating rate

The evaporation is the most important mechanism for heat dissipation from animal body. The body excess heat can be dissipated through cutaneous sweating (Gebremeghin and Wu, 2001). This process is influenced by weather parameters such as wind velocity, air temperature, relative humidity and thermal and solar radiation. Sweating, as panting, are two automatic primary responses of an animal under heat stress (Gaughan and Cawdell-Smith, 2015). In wool seep sweating is much less important than respiratory evaporation because of the presence of a wool coat.

However, sheep can also dissipate heat from the surface of the body as a result of water diffusion through the skin as insensible perspiration (Marai et al., 2007). In goats, on the opposite, the sweating is more important than in sheep. Goats are not limited by a wool coat and in a study during a 90 min period of heat exposure, hydrated goats increased evaporation by both panting and sweating (Baker, 1989). When respiratory mechanism fails to maintain the homeostasis, it active the evaporative heat loss by increasing sweating rate about 70-85% (Mili and Chutia 2021). The great characteristic of goats is the greater sweating rate and the lower body weight-surface ratio, which allows efficient way to heat dissipation from the body to the surrounding environment (Salama et al., 2014).

Feed and water intake

A heat stress condition influence feed intake in goats (Sano et al., 1985) and ewes (Abdalla et al., 1993) reducing intake, digestibility and utilization efficiency (Hirayama et al., 2004; Popoola et al., 2014). The decrease of feed intake is connected to the attempt to create less metabolic heat because, especially in ruminants, the increment of feeding produces an important quote of heat (Kadzere et al., 2002). Exposure of sheep to heat stress influences the metabolism of protein, energy and mineral balance, enzymatic reactions, hormonal secretions and blood metabolites. A high presence of roughages in diets aggrieves the situation due to high metabolic effort to process the fiber with rumination and mastication and to the fermentation heat at rumen level. Thus it’s possible to decrease dietary fiber to reduce heat stress in summer. Even though other studies showed the ability of ruminants to better digest roughage increases in warmer temperatures and than in colder environments (Conrad,1985). Although goats have a better adaptation in convert poor quality feeds to good products in rangelands, a prolonged heat stress condition create a severe discomfort. A study of Hamzaoui et al.

(2013), establish that heat stress cause in goats a 27% reduction of DMI from day 1 of experiment to day 19, and a 14% reduction from day 20 to 35, underlining a gradual adaptation of animas to this several conditions. In the particular sheep case, many studies show a significantly feed intake decrease during heat stress conditions in Croix, Karakul, Rambouillet breeds (Monty et al., 1991), Sardinian and Comisana breeds (Nardone et al., 1991). However, it’s possible to modify the rations in order to reduce the negative effect of heat stress. These adjustments may include changes in feeding schedules, grazing time and diet composition such as: dietary NDF adjustment, the use of high-quality fibre forage, increased energy density (protected fat or concentrates) and using feed additives (sodium bicarbonate, niacin, antioxidants, fungal culture, yeast culture) (Al Dawood, 2017) and other bioactive compounds helping vasodilatation. In grazing animals, in extensive conditions, it's important the provision of shade shelter (Silanikove, 2000). It has been observed that grazing time in sheep (minute of grazing/hour of the day) was significantly higher from 7:00 to 11:00 than 11:00 to 15:00 and from 15:00 to 19:00 during the day and in winter (20.1-11.4 °C), than in summer (35.4-18.9 °C); on the contrary water intake was significantly higher at 11:00 to 15:00 and 15:00 to 19:00 than 7:00 to 11:00 (in particularly in summer) (Marai et al., 1997; Marai et al., 2000). Khan end Gosh (1989) noted that consumption of water in sheep is 9-11% of total body weight during the winter and 19-25% during the summer. This aspect underlines the increasing of water intake, and so a water turnover during high stress conditions. In goats it has been observed that a restriction about 50 and 25% of water intake (compared to the ingestion recorded during the initial control period) causes a reduction of 20 and 18% respectively of milk production (Alamer, 2009). Furthermore, in the same study milk fat percentage was lower in goats that received 25% restriction while it was maintained unchanged in the 50% restriction group. Water is one of the most important nutrients required for the maintenance of life and generally sheep and goats consume less water per unit of DMI than cattle.

Milk production

Usually, as a consequence of reduced feed intake, in heat stress conditions milk production and quality decrease (Figure 5). In Saanen dairy goat exposed to a moderate or severe heat stress for 4 days, Sano et al. (1985) observed that animals lost milk production by 3 or 13% respectively. Brown et al. (1988) in another experiment observed that in dairy goats exposed to moderate heat stress condition for 5 weeks (34 °C and 25% humidity; THI 79), lost milk production in Alpine goats but not in Nubian goats underlining the difference level of tolerance between breeds. Hamzaoui et al. (2012) find another aspect that influence the milk yield loses, that is to say the stage of lactation. In fact, early lactating dairy goats suffered greater milk yield losses (−9%) compared to late lactating animals (−3%). This is determined by an increase maintenance requirement of animals necessary for extra activities (muscle movements for panting, greater sweating, increased chemical reactions in the body, and the production of heat stock pretend that consumes large amounts of ATP) that an insufficient feeding can’t satisfy. The increasing of maintenance requirements due to heat stress is estimated to 30% by NRC (2007).

About quality milk, studies discovers that dairy goats exposed to heat stress conditions decrease their milk protein production from -6 to -13% and lactose production from -1% to -5% (Brasil et al., 2000; Hamzaoui et al., 2013). According to Joshi et al. (1968), milk protein synthesis can be influenced by increased sweat secretion that contains protein and urea and by decreased protein intake under heat stress, that limits the availability of amino acids. In sheep, a significant and marked reduction of milk production has been observed by a study of Peana et al. (2007a). When maximum and mean temperatures were higher than 21-24°C and 15-21°C respectively, animals' losses up to 15% of milk production (0.30 kg/d per head) and when minimum temperatures changed from 9-12°C to 18-21°C, the reduction was about 20% (0.39 kg/d per head). A decrease of 20% (0.38 kg/d per head) was also observed when THI passed from 60-65 to 72-75. This marked effects of THI on milk yield, is considering much challenging in sheep than in dairy cattle (Nardone et al., 1992; Armstrong, 1994) even if dairy sheep and smaller size animals should be less sensitive than larger ruminants to heat stress based on the surface/volume ratio favouring higher heat dissipation. Peana et al. (2007a; 2007b) showed that milk yield was negatively affected when THI was above 65; Finocchiaro et al. (2005) observed a decrease in milk yield when THI was above 73; Sevi et al. (2001) observed a decrease in milk yield when THI was above 81. However, two particularly condition influenced the recovery tendence of milk loss: i) wind speed, it might increases 10% in milk yield when speed increase from 1.5-2.5 m/s to 2.5-4 m/s, taking out hot-humid air from the stable and ii) the increase of mean air humidity if temperature iof the air is not considered (Peana et al., 2007a). Milk yield increased of 10% with air humidity higher than 45-55%, with additional increase with relative humidity higher than 75-85%. The same authors observed minimum effects on milk composition except for milk somatic cell count. In fact, it's been observed that when maximum temperature varied from 21-24 °C to 33-36 °C, the somatic cell count increased from 236'000 to 375'000.

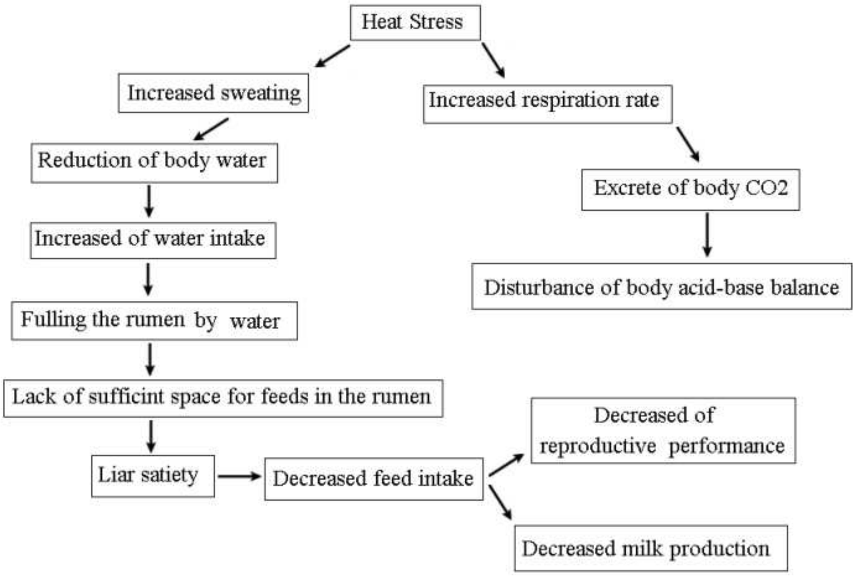

Figure 5. Some important results of heat stress in dairy cows (Atrian and Shahryar, 2012).

1.6. Relationship between surface, volume, and heat dissipation

The growth, milk production, pregnancy and activity are important components of metabolic heat production. But also, one is an important factor: the exchange of heat between animal and environment. The interphase of the animal for ski convective, radiant, and evaporative heat loss is the surface area (SA), complemented by convective and evaporative heat loss via the respiratory system. The body surface is an important component of heat exchange models and it is also used in energy needs calculation models (ARC, NRC, CNCPS) during cold and heat stress conditions. During time, several studies have formulated predictive equations that relate body surface to body mass. Equations used to presume similarity of body proportions imply that body shape and body mass density, remain similar with increasing body mass and imply similarity of body portions with increasing body mass. All of the equations used, share the same exponent (0.67) for body mass. They differ only in the factor: Meeh's with 0.105; Brody's 0.14; Johnson's 0.235. Mitchell used a 0.085 factor derived from measurements of sheared sheep (BW between 24 to 38 kg). After, this factor was reduced to 0.09.

Putting in relationship these equations in cattle, the body surface area estimates are similar when the body weight below 100 kg; however, they diverge progressively with weight gain. The body surface area calculated with Meeh equation estimates markedly higher than those obtained with the other equations. Within a species, body mass increases during growth and aging. The increase of body weight is associated with changes in the proportion of body parts, mainly in the relative reduction of body appendages, which would reduce surface area per unit of body weight. The consideration of growth and aging can add precision to equations based on direct measurements of the surface area of animals of different weights within a species. A weak point is the fact that equations are based in measurements made on animals 50-60 years ago, with animals they had different size to genetic and environmental changes. Today animals' size is increase and it is therefore possible that relationship between surface area and body mass has been altered. Basal metabolism had been related in the past to body surface area, considered proportional to live weight$^{0.67}$ (Blaxter, 1989; Berman, 2003). Most feeding systems express the metabolizable energy requirements as funcion of live weight$^{0.75}$ This relationship between body surface area and body weight (BW) is controversial and it may reduce the applicability of these equations (Berman, 2003). It appears that the mathematical association between BW and basal metabolism is empirical and thus it is affected by many different factors, such as species, age, body surface, body insulation, mass of visceral organs, and diets used. As more mechanistic and dynamic approaches and models are developed, the importance of scaling heat energy on body weight will be reduced (Cannas et al., 2010). It suggest that volume to surface rate and scaling factors are very important for the heat dissipation rates.

1.7. Technical practices to mitigate heat stress

Adequate nutritional strategy should be followed to meet the energy requirements of heat stressed sheep and to remedy the shortcoming of the heat-induced oxidative stress and to facilitate vasodilatation. In this way, rations rich in low fermentable proteins and fats may be administered, in combination with controlled quantities of concentrate to avoid acidosis. It is importanti to use high energy diets to balance the decrease of DMI and the increasing energy demand for thermoregulation (Sevi et al., 2012). Fiber must have good quality (Slimen et al., 2019). Bioactive compounds can be also used with high antioxidant or vasodilatation properties. A summary of nutritional approaches in ruminants is reported in Table 2. The implementation of these techniques also might ensure health to animals and humans (just related to the risk of hypercholesterolemia due to high levels of lauric+myristic+palmitic acids in milk).

The use of shades, feeding and grazing strategies (feeding time and planning), providing water, handling time, the use of fans and evaporative cooling, and site selection of animals' housing is also beneficial (Al Dawood, 2017).

Traditional heat stress alleviation with managerial actions have included providing shade and/or sprinklers and genetic selection of heat-tolerant breeds (Godfrey et al. 2013). Current research tends to suggest that the nutritional management of sheep (Sevi and Caroprese 2012; Peana et al., 2007a) and dietary supplements have become focal areas for heat stress alleviation. Several strategies were summarize by Lees et al. (2017; Figure 6) and some examples are also reported in Table 2. From the point of view of the farm facilities, heat stress can be limited through the installation of cooling systems in the barn such as fans and gargoyles, but also through the opening of roofs and walls to ensure adequate ventilation for the animals. Better ventilation inside the barn removes moisture, odor, and keeps the floor dry; then modifying the environment by increasing air movement improves its flow over the animals' bodies. However, when the skin temperature exceeds the ambient temperature, the animal is no longer able to dissipate heat through convection (Silanikove, 2000). It is important to provide adequate shade with trees or sheds thus reducing the animals' exposure to direct solar radiation despite the fact that environmental temperature or relative humidity cannot be altered (Gaughan et al. 2004b). Shaded areas and changes in microclimate can potentially reduce the thermoregulation mechanisms evoked by sheep, thereby reducing the energy demands associated with them. The availability of fresh water at reasonable distances should also be ensured to reduce the movement of sheep during the hottest hours. Another key aspect is shearing; in fact, this increases the rate of heat exchange between the sheep and their surroundings. Numerous authors have indicated that sheared sheep have lower body and rectal temperatures than non-sheared sheep, as well as a lower respiratory rate. The potential effects of climate change are difficult to quantify due to the complex relationship that exists between animals and their environment. The impact of climate change on animal production may be further confounded through performance-based selection of livestock. However, by selecting animals with superior productivity, there is the potential that these selection pressures may increase an animal’s susceptibility to heat stress, due to the relationship that is observed between animal productivity and metabolic heat production (Rhoads et al. 2013). The implications of forecasted climate change on heat stress in sheep are particularly concerning for developing nations (Marino et al. 2016). With the implementation of management strategies, producers are able to reduce the impact of heat stress. Better management of sheep, and other species, may reduce the initiation of thermoregulatory mechanisms which allows for better energy utilisation for growth and/or production. In the face of climate change, the continued development of heat stress management tools is needed to ensure the sustainability of animal-based agricultural enterprises.

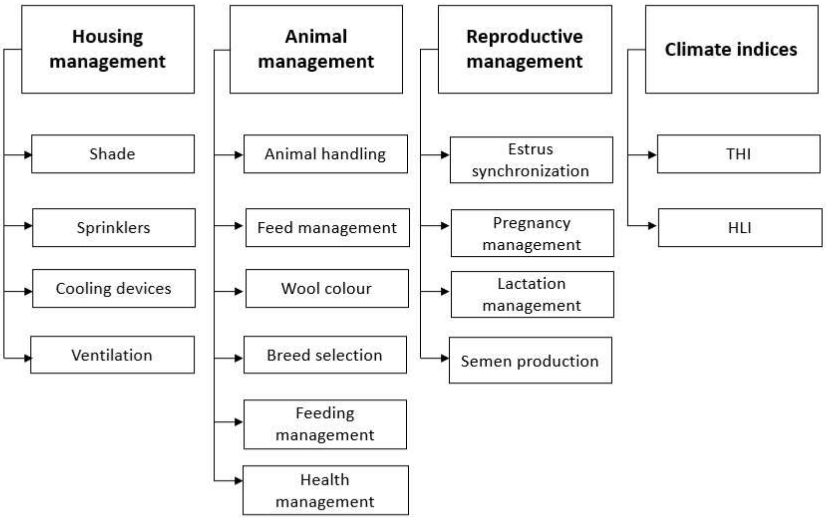

Figure 6. Management strategies to mitigate heat stress. Adapted From Lees et al. (2017).

Table 2. Examples of techniques to contrast heat stress.

Species	Treatments	Effects	Study
Cows	Protected fat	Increase of MY	Roskopf et al., 2023
Cows	*Chromium* yeast	Reduction of RT and RR and increase of DMI and milk lactose content	Shan et al., 2020
Cows	*S. Cerevisiae* yeast	Increase of MY and of DMI conversion efficiency (+7.4%)	Perdomo et al., 2019
Goats	Fat (+4%)	Reduction of RT	Sarangi, 2018
Sheep	Selenium-yeast	Improve reproductive performance	De et al., 2017
Lambs	Antioxidants	Oxidative status improvement	Chauhan et al., 2014
Cows	Encapsulated niacin	Reduction of VT and increase of evaporative heat lost	Zimbelman et al., 2010/2014
Sheep	Whole flaxseed	Reduction of RR	Caroprese et al., 2012
Sheep	Shade	Reduction of RT and BCS loss	Sevi and Caroprese, 2012
Goats	Soybean oil	Increase milk fat content	Salama et al., 2012
Sheep	Yeast (5g/d)	Improve reproductive traits, blood components and thermo-cardio respiratory activities (pre-mating/gestation)	Abdel Rahman et al., 2012
Goats	Shade	Reduction of RT and RR	Hammadi et al., 2012
Goats	Vitamin C and E	Reduction of excessive production of free radicals	Silanikove et al., 2010

Species	Treatments	Effects	Study
Goats	Vitamin C and E	Reduction of TR and RR	Sivakumar et al., 2010
Goats	Ventilation (1h/d)	Increase of DMI (+18%), water intake (+7%) and MY (+21%)	Darcan and Güney, 2008
Sheep & Goats	Shade	Improvement in weight gain, MY and reproduction	Berger et al., 2004
Sheep	Shade	Reduction of unsaturated (-4%) and saturated (-13%) acids in milk	Sevi et al., 2002
Sheep	Ventilation (173m^3/h/ewe)	Reduction of total coliform counts	Sevi et al., 2002
Sheep	Ventilation (70m^3/h/ewe)	Improvement of MY and quality	Sevi et al., 2002
Sheep	Shade	Reduction of lauric, myristic and palmitic acid content in milk	Grummer, 1991
Cows	Sodium Bicarbonate	Increase of DMI and MY	Schneider et al., 1984

1.8. Methane emissions and environmental impact

Climate change related problems are also usually connected to livestock contribution to global warming which mainly depend from methane emissions. Thus among farm good practices, methane emission mitigation strategies and tecniques for adaptation to climate change are two very tight issues. Methane (CH_4) is the second most important greenhouse gas emitted from anthropogenic fount (Wuebbles and Hayhoe, 2002; IPCC, 2006). One quarter of this quote comes from ruminants activities (Beauchemin et al., 2008). As early as 2005 with the entry into force of the Kyoto protocol, the signatories committed themselves to reducing greenhouse gas emissions to an agreed level (Kyoto protocol, 2009), with the aim of reducing the overall production of CH4 production from ruminants (Ellis et al., 2007), making a major contribution to climate change and global warming (Boadi et al., 2004).

The global livestock sector is responsible for 14.5% of anthropogenic greenhouse gas emissions (Gerber et al., 2013). In 2020 these amounted to 380 Mt, of which 49 Mt/year came from livestock (United Nations Environment Program and Climate and Clean Air Coalition, 2021). Enteric fermentation alone is responsible for the release of 87-97 Tg of methane into the atmosphere on an annual basis (Chang et al., 2019). The 38.6% of total agricultural methane emissions derive from enteric livestock emissions (FAOSTAT, 1961-2019 http://www.fao.org/faostat/en/#data/GE; Figure 6).

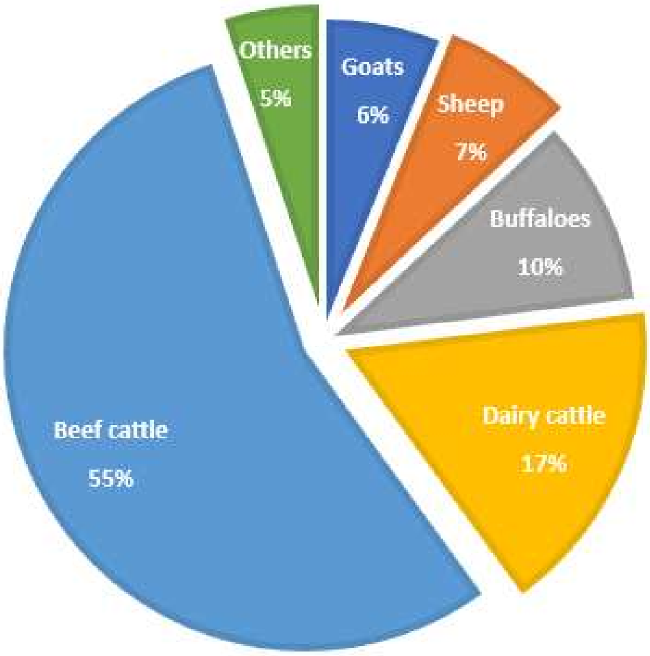

Figure 7. Contributions of different animal species to the total CH4 emissions from livestock (Zhao et al., 2022).

In 2015 at the United Nations Climate Change Conference held in Paris a collective commitment was established to keep global warming "below 2" (United Nations Framework Convention on Climate Change, 2015). Current emissions (primarily CO2 and secondarily CH4) are expected to increase global temperatures by 1.5°C within the next 15 years and by 2°C within 35 years (Howarth, 2015).

The CH4 emitted by ruminants (Figure 7), in addition to therefore representing a threat to the ecosystem, also represents a great energy loss for the animal. The main factor influencing production levels is feed intake and its composition. CH4 emissions are strongly correlated with feed intake (Moorby et al., 2015) and dietary lipids administered to animals (Beauchemin et al., 2008; Knapp et al., 2014). Forage proportion is an important factor influencing CH4 production, which can be reduced by feeding higher levels of concentrates in diets (Johnson and Johnson, 1995, cited in Ramin and Huhtanem, 2013). These factors can in fact determine a loss of energy which can vary from 2 to 12% of the gross energy intake (Guan et al. al., 2006), where each liter of methane takes 39.5 kJ of energy from the host animal; or even up to 15% of digestible energy intake (Steinfeld et al., 2006; Patra et al., 2012).

1.8.1. Rumen microbioma and methane production

The rumen is a methanogenic anaerobic environment in which gases such as CO_2 (65%), CH_4 (35%), H_2, Nitrogen, Oxygen are present (very low quantities) (Antonietta Guglielmetti, 2009). CO_2 and H_2 are produced in abundant quantities starting from the degradation of the administered diets. Over the years, various studies have deepened the knowledge relating to the microbial composition of the rumen, thus managing to better clarify the mechanisms that develop internally. Inside the rumen we find 3 large groups of microorganisms: Bacteria, Fungi, Protozoa. Bacteria are the largest, most diverse and metabolically active group (Deusch et al., 2017) among other rumen microbes and account for approximately 50-70% of the rumen microbial population with $1x10^{10}$–10^{11} bacterial cells per gram of rumen content (Newbold and Ramos-Morales, 2020; Matthews et al., 2019). It is estimated that there are over 7,000 species of bacteria representing over 19 different phyla in the rumen (of which Firmicutes and Bacteroidetes predominate, followed by Proteobacteria; Sanjorjo et al., 2023). Fungi represent about 10-20% of the ruminal microbiome and the predominant phyla is the Neocallimastigomycota. Finally, the protozoa representing up to 50% of the rumen biomass (Newbold et al., 2015; Newbold et al., 2022) and are divided in ciliated (the most numerous and strictly anaerobic) and flagellates. They are contained in the rumen respectively $1x10^4$-10^6 bacterial cells per ml and $1x10^3$-10^5. Ciliated protozoa are divided into Holotrich and Entodyniomorphidae. The first have cilia on the peristome, the latter are entirely covered by them (Coleman, 1980; cited by Sanjorjo 2023) and it is the most predominant (up to 90% of total protozoa).

Rumen protozoa are responsible for protein breakdown, bacterial predation (Williams and Coleman, 1992; cited by Thirumalaisammy et al., 2022), reduction of potential pathogen shedding (Newbold et al., 2015), lipid metabolism, and shifts in volatile fatty acid production (Eugène et al., 2004). They transfer hydrogen to other microorganisms, in particular to methanogens (Li et al., 2018) belong to the phylum Euryarchaeota (Balch et al., 1979), which constitute 3-4% of the rumen microbiota (Yanagita et al., 2000). In this way they contribute significantly to the mechanism of CH_4 production in the rumen (Figure 8), a condition confirmed by defaunation tests (Tan et al., 2020).

As already mentioned, there are three main substrates used for the production of methane: CO_2, compounds containing a methyl group and acetate (Figure 9 and 10; Liu and Whitmen, 2008). The most important pathway in the rumen is the hydrogenotrophic one which uses CO_2 as a carbon source and H_2 as an electron donor (Hungate, 1967; cited by Morgavi et al., 2010). Also formate (produced by protozoa (Tokura, 1997; cited by Morgavi et al., 2010) is an important electron donor used by hydrogenotrophic methanogens of the rumen (they can represent up to 18% of enteric methane; Hungate, 1970; cited by Morgavi et al., 2010). Methylamines and methanol enterics can be used by methylophrophic methanogens (Methanosarcinales and Methanobacteriales). Another important route is the one using acetate: acetyclastic route (limited only to methanogens belonging to the order Methanosarcinales; Liu and Whitman, 2008).

For this reason, methanogens associated with rumen protozoa are responsible for 37% of enteric methane emissions (Machmüller et al., 2003). In fact, several studies have shown that the number of protozoa present in the rumen is linearly related to the volume of enteric methane emissions, even if not all methanogenesis is dependent on the activity of the protozoa (Guyader et al., 2014). Defaunation, specifically, it consists in the removal of protozoa from the rumen using a wide variety of physical and chemical techniques (Morgavi et al., 2010). In a series of studies it has been observed that defaunation is able to reduce methanogenesis up to 11% and increase microbial intake up to 30% (Newbold et al., 2015). In other studies it has been observed that its application has increased the molar proportion of propionate in the rumen and decreased that of butyrate and acetate (Morgavi et al., 2010), a condition favorable to the reduction of enteric methane due to the lack of H_2 as a substrate for methanogenesis (Demeyer, 1991; cited by Morgavi et al., 2010). An elaborate data analysis conducted by Morgavi et al., (2010) on measurements of entheric methane carried out in a series of experimental tests, indicated that a reduction of 0.6 g of methane/kd DMI corresponds to a reduction of 105 protozoan cells/ml of rumen content.

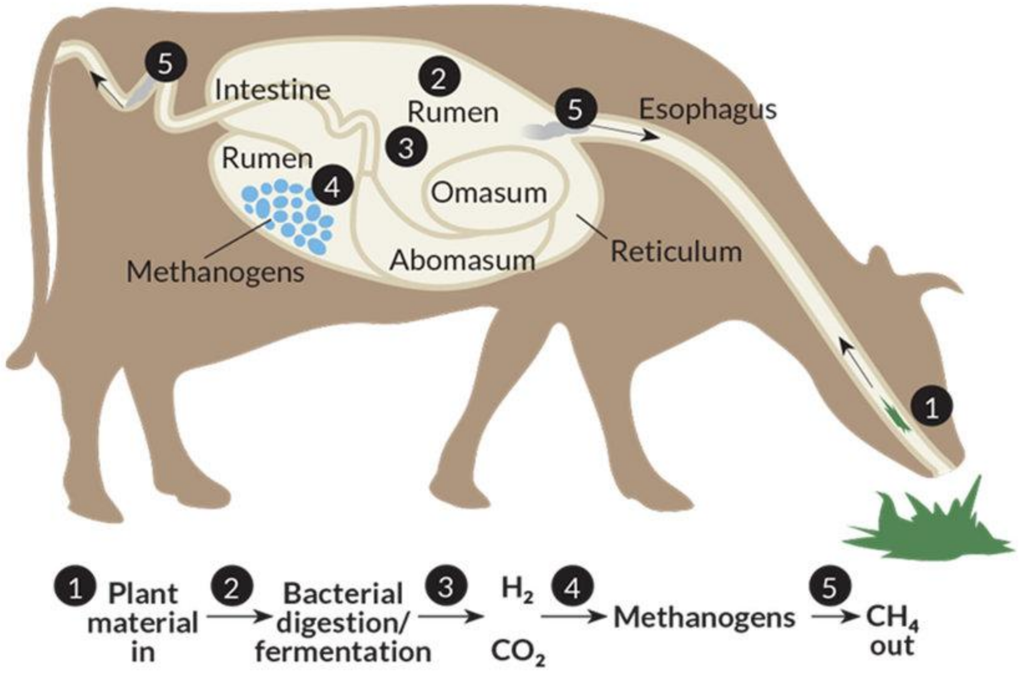

Figure 8. Methane production in ruminants (adapted from Zhao et al., 2020).

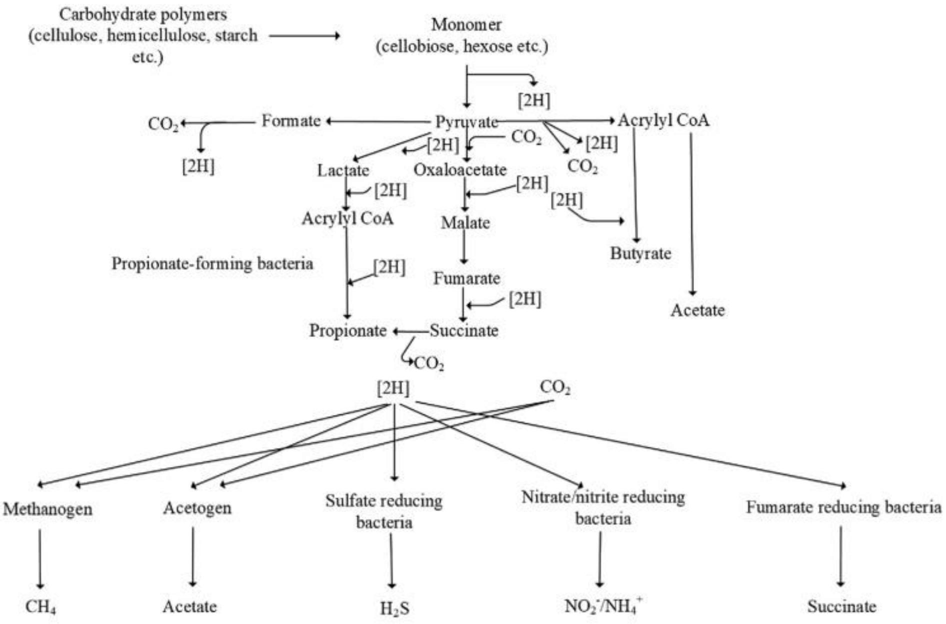

Figure 9. Biochemistry of ruminal fermentation of carbohydrates and H_2 disposal ways. Modified based on Lan and Yang, 2019 (cited by Zhao et al., 2022).

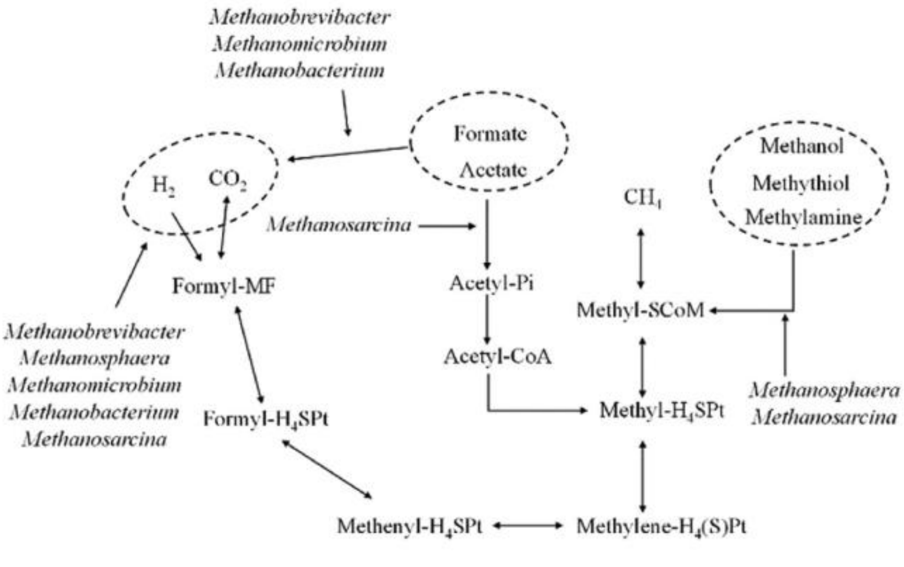

Figure 10. Simplified methanogenesis pathways and related methanogens genera in the rumen. Modified based on Honan et al., 2021 (cited by Zhao et al., 2022).

1.8. Good practices to mitigate methane emissions

The particular characteristic of CH_4 of having a relatively short lifetime in the atmosphere (8.4-12 years; Ehhalt et al., 2001) is of great help in achieving the collective goal of stabilizing the climate (Reisinger et al., 2021). it is possible to follow two paths to achieve the result: studying nutritional strategies and/or improving the management strategies of farms (genetics, reproduction, productivity, pasture management, stock numbers, etc.). The nutritional approach has been widely discussed and studied over the years. A number of additives have been developed with the ability to reduce CH4 emissions: essential oils, tannins, saponins, nitrates, protozoa-free, lipids, bacteriocins, phytochemicals, 3-nitrooxypropanol, acetogens, organic acids, ionophores, algae (Torres et al., 2023; Almeida et al., 2021; Beauchemin et al., 2009; Chung et al., 2011; Moate at al., 2011; Table 3).

Over the years there has been a growing interest in the use of secondary plant metabolites with the aim of improving livestock productivity while reducing the environmental impact (Cieslak et al., 2012; Chen et al., 2015). An example of secondary plant metabolite, important for its wide application in animal production are condensed tannins (high molecular weight water-soluble polyphenolic compounds capable of modulating rumen fermentation (Hristov et al., 2013; Hoehn et al., 2018). It has already been found in the past that condensed tannins can bind with proteins in feed, saliva, tissues, enzymes and microbes with a consequent reduction in the degradability of rumen proteins and a reduced loss of urinary nitrogen (Dentinho et al., 2014; Henke et al., 2017). Other effects found are the control of swelling and intestinal parasites (Naumann et al., 2014) in ruminants and the important reduction of enteric emissions of methane (Adejoro et al., 2019; Hassen et al., 2016; Carulla et al., 2005). In fact, condensed tannins directly inhibit the growth of methanogens through the tanning action of their functional proteins, with consequent bacteriostatic and bactericidal effects (Field et al., 1989 cited by Adejoro et al., 2019; Tavendale et al., 2005). However, incorrect use of this supplement can lead to a reduction in DMI, in the digestibility of nutrients (Hristov et al., 2013; Mueller-Harvey, 2006) and in their own absorption (Wahorn, 2008; Dschaak et al., 2011) with moderate and high doses. No less important is the astringent power of the compound itself (Fernàndez et al., 2012; Patra et al., 2011). A valid method to overcome this kind of problem can be the encapsulation of the additive (not only in the case of tannins), guaranteeing greater control of its release in the required site, optimizing its use (Munin end Edwards-Lévy, 2011; Adejoro et al., 2018; Wood et al., 2009).

Other promising ingredients to increase diet density and mitigate methane production are oil (Vargas et al., 2020) and lipids (Morgavi et al., 2010). Commonly used edible oils are for example: linseed, canola, palm and canola. Silkworm Pupae Oil for example, contains good amounts of unsaturated and saturated fatty acids (Thirumalaisamy et al., 2020). Polyunsaturated fatty acids carry out the activity of reducing the quantity of protozoa present in the rumen, thus managing to reduce the production of enteric methane (Guyader et al., 2017).

Methane inhibition is generally short-lived as the rumen is highly adaptable (Mathison et al., 1998; cited by Thirumalaisamy et al., 2022). Indeed, by blocking the administration of a supplement, animals return to normal methane emissions (Hristov et al., 2013). The use of phage immunization vaccines is also being studied (Kumar et al., 2014).

Table 3. Examples of techniques to mitigate methane emissions.

Species	Treatments	Effects	Study
Sheep	*Candida Tropicalis* ($4x10^8$ CFU/d per head)	Reduction of CH_4 emissions and Nitrogen excretions	Liu et al., 2023
Sheep	a. Lipids b. Oils c. Tannins	a. Reduction in 6.28g/d of CH_4 and 5.87 g CH_4/kg of DMI b. Reduction of CH_4 c. Reduction in 1.22 g/d of CH_4 and 2.61 g CH_4/kg of DMI	Torres et al., 2023 (meta-analysis)
Sheep (male)	Silkworm Pupae Oil	Reduction of CH_4 emissions and increase of body weight gain	Thirumalaisamy et al., 2022
Ruminants	Seaweed, 3-NOP and NO3-	The most effective feed additives for CH_4 abatement	Almeida et al., 2021 (meta-analysis)
Cows	Mealworm and cricket oil (5% of DM)	Reduction of CH_4 emissions without altering VFA concentration	Jayanegara et al., 2020
Sheep	a. Tannins (30g/kg DM) b. Soybean oil (50g/kg DM) c. Tan+Soyb (30-50g/kg DM)	a. Absence of decrease of digestibility b. Decrease of ADF and NDF digestibility and reduction of methane emissions c. Decrease of OM and NDF digestibility and reduction of methane emissions The only Soybean oil is sufficient to reduce methane production	Lima et al., 2019
Sheep	Crude Acacia tannin exctact and Lipid encapsulated Acacia tannin extract	Reduction of CH_4 emissions, g/d (-32% Crude tannin; -25%Lipid encaplusalted); g/kg DMI (-30% Crude tannin; -19%Lipid encaplusalted).	Adejoro et al., 2019

Species	Treatments	Effects	Study
Sheep	Essential oils (+200mg; +400mg)	Reduction of CH_4 emissions (-24,5 l/kgDOM with 200mg; -27.6 l/kgDOM with 400mg)	Soltan et al., 2018
Species	Treatments	Effects	Study
Heifers	*Leucaena leucocephala* and *Pennisetum purpureum* (rispectively 80-20% of DM)	Reduction of CH_4 emissions (about 61%)	Piñeiro-Vázquez et al., 2018
Lambs	Condensed tannins (*Ficus infectoria* and *Psidium guajava* leaf meal mixture) at 1-2% of diet	Improving nitrogen metabolism, growth performance, wool yield, feed conversion ratio and reduced methane emission.	Pathak et al., 2017
Sheep (male)	Tanniniferous tropical tree leaves (*Ficus benghalensis, Artocarpus heterophyllus and Azadirachta indica*)	Reduction of entodinimorphs protozoa and consequently CH_4 emissions	Malik et al., 2017
Sheep	Sodium Nitrate + Sulphure (rispectively 5+04% of DM)	Reduction of CH_4 emissions (-18.2%)	Arif et al., 2016

Species	Treatments	Effects	Study
Goats	Sodium Nitrate + Sulphure (rispectively 5+04% of DM)	Reduction of CH_4 emissions (-19.6%)	Arif et al., 2016
Cows	Sanguisorba officinalis tannin extract (40 and 100mg)	Reduction of CH_4 emissions (in vitro dry matter digestibility and of protozoal population only with the highest dose: 100 mg)	Cieslak et al., 2016
Sheep	Purified hydrolysable (chestnut and sumach) and condensed tannins (mimosa and quebracho) +1 mg/ml	Reduction of CH_4 emissions, ranged from 22.3 to 36.7%. Hydrolysable tan. had a greater effect than condensed tan.	Jayanegara et al., 2015
Sheep	Nitrate (+4% KNO_3)	Reduction of CH_4 emissions	Nolan et al., 2010
Ruminants	Defaunation	Reduction of CH_4 emissions (main of -10.5%)	Morgavi et al., 2010
Sheep	Nitrate (+2.6% DM) and Sulfate (+2.6% DM)	Reduction of CH_4 emissions, L/d (-32% nitrate treat., -16% sulfate treat.). Reduction of Oxigen consumption (-7% nitrate treat.) and of Carbon dioxine production (-6% nitrate treat.). Opposite results for the sulfate treatment.	van Zijderveld et al-. 2010
Sheep	Eucalyptus essential oils (+10 and + 20ml/d per head)	Reduction of CH_4 emissions (-31% with 10ml; -22% with 20ml)	Sallam et al., 2009
Sheep	Defaunation (Protozoa-free)	Reduction of CH_4 emissions (about -20%)	Morgavi et al., 2008

Species	Treatments	Effects	Study
Sheep	Tea saponin (5g/kg DM) and Tea saponin plus disodium fumarate (20g/kgDM)	Reduction of CH_4 emissions (-8.5% TS; -9.6% TS plus disodium fumarate)	Yuan et al., 2007

1.9. References

Abdalla, E.B., Kotby, E.A., Johnson, H.D. 1993. Physiological responses to heat-induced hyperthermia of pregnant and lactating ewes. Small Rumin. Res. 11, 125–134. https://doi.org/10.1016/0921-4488(93)90145-8

Abdel Rahman, H., Baraghit, G.A., Abu El-Ella, A.A., Omar, S. S., Abo Ammo, F.F., Kommona, O.F. 2012. Physiological responses of sheep to diet supplementation with yeast culture. Egypt. J. Sheep Goats Sci. 7 (1), 27–38. 10.21608/EJSGS.2012.27017

Aboul-Naga, A.I. 1987. The role of aldosterone in improving productivity of heat-stressed farm animals with different techniques. Ph.D. thesis. Faculty of Agriculture, Zagazig University, Zagazig, Egypt.

Adejoro, F.A., Hassen, A., Thantsha, M.S. 2018. Preparation of acacia tannin loaded lipid microparticles by solid-in-oil-in-water and melt dispersion methods, their characterization and evaluation of their effect on ruminal gas production In Vitro. PLoS ONE 13, e0206241. https://doi.org/10.1371/journal.pone.0206241

Adejoro, F.A., Hassen, A., Akanmu, A.M. 2019. Effect of Lipid-Encapsulated Acacia Tannin Extract on Feed Intake, Nutrient Digestibility and Methane Emission in Sheep. Animals. 9, 863. Adejoro, F.A.; Hassen, A.; Thantsha, M.S. Characterization of starch and gum arabic-maltodextrin microparticles encapsulating acacia tannin extract and evaluation of their potential use in ruminant nutrition. Asian-Australas. J. Anim. Sci. 32, 977–987. https://doi.org/10.3390/ani9110863

Alamer, A. 2009. Effect of water restriction on lactation performance of Aardi goats under heat stress conditions. Small Rumin. Res. 84, 76–81. https://doi.org/10.1016/j.smallrumres.2009.06.009

Al Dawood, A. 2017. Towards heat stress management in small ruminants – a review. Anim. Sci. 17, 59–88. https://doi.org/10.1515/aoas-2016-0068

Alexiev, J., Gudev, D., Popova-Ralcheva, S., Moneva, P. 2004. Thermoregulation in sheep. IV. Effect of heat stress on heart rate dynamics in shorn and in shorn ewes from three breeds. J. Anim. Sci. 41, 16–21.

Almeida, K.A., Hegarty, R.S., Cowie, A. 2021. Meta-analysis quantifying the potential of dietary additives and rumen modifiers for methane mitigation in ruminant production systems. Anim. Nutr. 7(4), 1219–1230. https://doi.org/10.1016/j.aninu.2021.09.005

Altınçekiç, Ş. Ö., Koyuncu, M. 2012. Çiftlik hayvanları ve stres. Hayvansal Üretim, 53(1), 27–37.

Arif, M., Sarwar, M., Mehr-un-Nisa, Hayat, Z., Younas, M. 2016. Effect of supplementary sodium nitrate and sulphur on methane production and growth rates in sheep and goats fed forage based diet low in true protein. J. Anim. Plant. Sci. 26(1), 69–78.

Armstrong, D.V. 1994. Heat stress interaction with shade and cooling. J. Dairy Sci. 77, 2044–2050. https://doi.org/10.3168/jds.S0022-0302(94)77149-6

Atrian, P., Shahryar, H.A. 2012. Heat Stress in Dairy Cows (A Review). Zool. Res. 2(4), 31–37. DOI: 10.5923/j.zoology.20120204.03

Baker, M.A. 1989. Effects of dehydration and rehydration on thermoregulatory sweating in goats. Physiol. J. 417, 421–435. https://doi.org/10.1113/jphysiol.1989.sp017810

Balch, W. E., Fox, G. E., Magrum, L. J., Woese, C. R., and Wolfe, R. S. 1979. Methanogens: reevaluation of a unique biological group. Microbiol. Rev. 43, 260–296. doi: 10.1128/mmbr.43.2.260-296.1979

Beauchemin, K. A., M. Kreuzer, F. O'Mara, and T. A. McAllister. 2008. Nutritional management for enteric methane abatement: A review. Aust. J. Exp. Agric. 48:21–27.

Beauchemin, K. A., S. M. McGinn, C. Benchaar, and L. Holtshausen. 2009. Crushed sunflower, flax, or canola seeds in lactating dairy cow diets: Effects on methane production, rumen fermentation, and milk production. J. Dairy Sci. 92, 2118–2127. https://doi.org/10.3168/jds.2008-1903

Berman, A. 2003. Effects of body surface area estimates on predicted energy requirements and heat stress. J. Dairy Sci. 86, 3605–3610. https://doi.org/10.3168/jds.S0022-0302(03)73966-6

Berger, Y., Billon, P., Bocquier, F., Caja, G., Cannas, A., McKusick, B., Marnet, P-G., Thomas, D. 2004. Principles of sheep dairying in North America. Cooperative Extension Publishing, A3767. University of Wisconsin-Madison, USA, 156 pp.

Blaxter, K.L. 1989. Energy metabolism in animal and man. Cambridge.

Boadi, D. A., K. M. Wittenberg, S. L. Scott, D. Burton, K. Buckley, J. A. Small, and K. H. Ominski. 2004. Effect of low and high forage diet on enteric and manure pack greenhouse gas emissions from a feedlot. Can. J. Anim. Sci. 84, 445–453. https://doi.org/10.4141/A03-079

Boldrini, J.L., Viana, M.P., dos Reis, S.F., Henning, B. 2018. A mathematical model for thermoregulation in endotherms including heat transport by blood flow and thermal feedback control mechanisms: changes in coat, metabolic rate, blood fluxes, ventilation and sweating rates. Lett. Biomath. 5, 129–173.

Brasil, L.H.A., Wechesler, F.S., Júnior, F., Gonçalves, H.C., Bonassi, I.A. 2000. Efeitos do Estresse Térmico Sobre a Produção, Composição Químicado Leite e Respostas Termorreguladoras de Cabras da Raça Alpina. Rev. Bras. Zootec. 29, 1632–1641.

Brockway, J.M., McDonald, J.D., Pullar, J.D. 1965. Evaporative heat-loss mechanisms in sheep. Physiol. J. 79, 554–568. 10.1113/jphysiol.1965.sp007680

Brown, D.L., Morrison, S.R., Bradford, G.E. 1988. Effects of ambient temperature on milk production of Rubian and Alpine goats. J. Dairy Sci. 71, 2486–2490. https://doi.org/10.3168/jds.S0022-0302(88)79835-5

Bruce J.M. 1993. Interactions between the animal and its environment. In Quantitative aspects of ruminant digestion and metabolism (ed. Forbes, J. M., France, J.). Scottish Agricultural College, Centre for Rural Building, Craibstone, Bucksburn, Aberdeen, UK, pp. 495-508.

Cannas, A., Atzori, A.S., Teixeira, IAMA, Sainz, R.D., Oltjen, J.W. 2010. The energetic cost of maintenance in ruminants: from classical to new concepts and prediction systems. In Energy and protein metabolism and nutrition (ed. Crovetto GM), pp 531–542.

Caroprese, M., Albenzio, M., Bruno, A., Annicchiarico, G., Marino, R., Sevi, A. 2012. Effects of shade and flaxseed supplementation on the welfare of lactating ewes under high ambienttemperatures. Small Rumin. Res. 102, 177–185. https://doi.org/10.1016/j.smallrumres.2011.07.010

Carulla, J.E., Kreuzer, M., Machmuller, A., Hess, H.D. 2005. Supplementation of Acacia mearnsii tannins decreases methanogenesis and urinary nitrogen in forage-fed sheep. Aust. J. Agric. Res. 56, 961–970.

Cieslak, A., Zmora, P., Pers-Kamczyc, E., Szumacher-Strabel, M. 2012. Effects of tannins source (Vaccinium vitis idaea L.) on rumen microbial fermentation in vivo. Anim. Feed Sci. Technol. 176, 102–106. https://doi.org/10.1016/j.anifeedsci.2012.07.012

Cieslak, A., Zmora, P., Matkowski, A., Nawrot-Hadzik, I., Pers-Kamczyc, E., El-Sherbiny, M., Bryszak, M., Szumacher-Strabel, M. 2016. Tannins from sanguisorba officinalis affect in vitro rumen methane production and fermentation. J. Anim. Plant. Sci. 26, 54–62.

Chang, J., Peng, S., Ciais, P., Saunois, M., Dangal, S. R. S., Herrero, M., et al. 2019. Revisiting enteric methane emissions from domestic ruminants and their δ13CCH4 source signature. Nat. Commun. 10, 1–14. doi: 10.1038/s41467- 019-11066-3

Chauhan, S.S., Celi, P, Leury, B.J., Clarke, I.J., Dunshea, F.R. 2014. Dietary antioxidants at supranutritional doses improve oxidative status and reduce the negative effects of heat stress in sheep. J. Anim. Sci. 92(8), 3364–3374. https://doi.org/10.2527/jas.2014-7714

Chen, D., Chen, X., Tu, Y., Wang, B., Lou, C., Ma, T., Diao, Q. 2015. Effects of mulberry leaf flavonoid and resveratrol on methane emission and nutrient digestion in sheep. Anim. Nutr. 1, 362–367. https://doi.org/10.1016/j.aninu.2015.12.008

Chung, Y. H., M. L. He, S. M. McGinn, T. A. McAllister, K. A. Beauchemin. 2011. Linseed suppresses enteric methane emissions from cattle fed barley silage, but not from those fed grass hay. Anim. Feed Sci. Technol. 166–167, 321–329. https://doi.org/10.1016/j.anifeedsci.2011.04.022

Coleman, G.S. 1980. Rumen ciliate protozoa. Adv. Parasitol. 18, 121–173. https://doi.org/10.1016/S0065-308X(08)60399-1

Conrad, J.H. 1985. Feeding of farm animals in hot and cold environments. In Stress Physiology in Livestock (ed. Yousef, M.K.). CRC Press, Inc., Boca Raton, Florida, U.S.A., Place, Corvallis, OR 97330, USA.

Curtis, S.E. 1981. Environmental Management in Animal Agriculture. Iowa State University Press, Ames, IA.

Darcan, N., Güney, O. 2008. Alleviation of climatic stress of dairy goats in Mediterranean climate. Small Rumin. Res. 74, 212–215. https://doi.org/10.1016/j.smallrumres.2007.02.007

Das, M.R., Sarma, R.K., Saikia, R., Kale, V.S., Shelke, M.V., Sengupta, P. 2011. Synthesis of silver nanoparticles in an aqueous suspension of graphene oxide sheets and its antimicrobial activity. Colloids and Surf. B: Biointerfaces 83, 16–22. https://doi.org/10.1016/j.colsurfb.2010.10.033

De, K., Sahoo, A., Shekhawat, I., Kumawat, P., Kumar, D., Naqvi, S.M.K. 2017. Effect of selenium-yeast feeding on amelioration of simulated heat stress and reproductive performance in Malpura ewe under semi-arid tropical environment. Indian J. Anim. Sci. 87(2), 163–167.

Demeyer, D.I. 1991. Quantitative aspects of microbial metabolism in the rumen and hindgut. In Rumen microbial metabolism and ruminant digestion (ed. JP Jouany), pp. 217–237. INRA Editions, Versailles, France.

Dentinho, M.T.P., Belo, A.T., Bessa, R.J.B. 2014. Digestion, ruminal fermentation and microbial nitrogen supply in sheep fed soybean meal treated with Cistus ladanifer L. tannins. Small Rumin. Res. 119, 57–64. https://doi.org/10.1016/j.smallrumres.2014.02.012

Deusch, S., Camarinha-Silva, A., Conrad, J., Beifuss, U., Rodehutscord, M., Seifert, J. 2017. A Structural and Functional Elucidation of the Rumen Microbiome Influenced by Various Diets and Microenvironments. Front. Microbiol. 8, 1605. https://doi.org/10.3389/fmicb.2017.01605

Dschaak, C.M., Williams, C.M., Holt, M.S., Eun, J.S., Young, A.J., Min, B.R. 2011. Effects of supplementing condensed tannin extract on intake, digestion, ruminal fermentation, and milk production of lactating dairy cows. J. Dairy Sci. 94, 2508–2519. https://doi.org/10.3168/jds.2010-3818

Ellis, J.L., Kebreab, E., Odongo, N.E., Mcbride, B.W., Okine, E.K., France, J. 2007. Prediction of methane production from dairy and beef cattle. J. Dairy Sci. 90, 3456–3466. https://doi.org/10.3168/jds.2006-675

Ehhalt, D., Prather, M., Dentener, F., Derwent, R., Dlugokencky, E., et al. 2001 Atmospheric Chemistry and Greenhouse Gases. In: Houghton, J.T., Ding, Y., Griggs, D.J., Noguer, M., van der Linden, P.J., Dai, X., et al., editors. Climate Change: The Scientific Basis. Contribution of Working Group I to the Third Assessment Report of the IPCC. Cambridge, United Kingdom. New York: Cambridge University Press. p. 239e87.

Eugène, M., Archimède, H., and Sauvant, D. 2004. Quantitative meta-analysis on the effects of defaunation of the rumen on growth, intake and digestion in ruminants. Livest. Prod. Sci. 85, 81–97. doi: 10.1016/S0301-6226(03)00 117-9.

Fahmy, S. 1994. Effect of crossing Romanov with Rahmani sheep on some physiological and productive performance. M.Sc. thesis. Faculty of Agriculture, Al-Azhar University, Cairo, Egypt.

FAO. 2022. Temperature Change. In: FAO. Rome. Cited March 2022. http://www.fao.org/faostat/en/#data/ET

Fernández, H.T., Catanese, F., Puthod, G., Distel, R.A., Villalba, J.J. 2012. Depression of rumen ammonia and blood urea by quebracho tannin-containing supplements fed after high-nitrogen diets with no evidence of self-regulation of tannin intake by sheep. Small Rumin. Res. 105, 126–134. https://doi.org/10.1016/j.smallrumres.2012.03.013

Field, J.A., Kortekaas, S., Lettinga, G. 1989. The tannin theory of methanogenic toxicity. Biol. Wastes. 29, 241–262. https://doi.org/10.1016/0269-7483(89)90016-5

Finocchiaro, R., van Kaam, J.B.C.H.M., Portolano, B., Misztal, I. 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

Gagge, A.P. and Gonzalez, R.R. 2010. Mechanisms of heat exchange: biophysics and physiology. Compr. Physiol. 45-84.

Gaughan, J.B., Mader, T.L., Holt, S.M., Lisle, A. 2008. A new heat load index for feedlot cattle. J. of Anim. Sci. 86, 226–234. Doi: 10.2527/jas.2007-0305

Gaughan, J., Cawdell-Smith, A.J. 2015. Impact of climate change on livestock production and reproduction. In Climate change impact on livestock: adaptation and mitigation (ed. Sejian, V., Gaughan, J., Baumgard, L., Prasad, C.). Springer, New Delhi, India, pp. 51–60.

Gebremedhin, K.G., Wu, B. 2001. A model of evaporative cooling of wet skin surface and fur layer. J. Therm. Biol. 26, 537–545. https://doi.org/10.1016/S0306-4565(00)00048-6

Gerber, P. J., Hristov, A. N., Henderson, B., Makkar, H., Oh, J., Lee, C., Meinen, R., Montes, F., Ott, T., Firkins, J. 2013. Technical options for the mitigation of direct methane and nitrous oxide emissions from livestock: a review. Animal. 7(2), 220–234. doi: 10.1017/S1751731113000876

Godfrey, R.W., Vinson, M.C., Ketring, R.C. 2013. The effect of a split feeding regimen and breed on body temperature of hair sheep ewes in the tropics, J. Anim. Sci. 91(11), 5202–5207. https://doi.org/10.2527/jas.2013-6559

Grummer, R.R. 1991. Effect of feed on the composition of milk fat. J. Dairy Sci. 74, 3244–3257. https://doi.org/10.3168/jds.S0022-0302(91)78510-X

Gupta, M., Kumar, S., Dangi, S.S., Jangir, B.L. 2013. Physiological, Biochemical and Molecular Responses to Thermal Stress in Goats. Int. J. Livest. Res. 3, 27–38.

Guan, H., Wittenberg, K. M., Ominski, K. H., and Krause, D. O. 2006. Efficacy of ionophores in cattle diets for mitigation of enteric methane. J. Anim. Sci. 84, 1896–1906. doi: 10.2527/jas.2005-652

Guglielmetti, A. 2009. Studio sulla produzione di metano nei ruminanti: valutazione in Vitro di alimenti e diete. University of Napoli, Federico II. PhD Thesis.

Guyader, J., Ungerfeld, E.M., Beauchemin, K.A., 2017. Redirection of metabolic hydrogen by inhibiting methanogenesis in the rumen simulation technique (RUSITEC). Front. Microbiol. 8, 393. doi: 10.3389/fmicb.2017. 00393

Guyader, J., Eugène, M., Nozière, P., Morgavi, D.P., Doreau, M., and Martin, C. 2014. Influence of rumen protozoa on methane emission in ruminants: a meta-analysis approach. Animal. 8, 1816–1825. doi: 10.1017/ S1751731114001852

Hammadi, M., Fehem, A., Harrabi, H., Ayeb, N., Khorchani, T., Salama, A.A.K., Casals, R., Such, X., Caja, G. 2012. Shading effects on respiratory rate and rectal temperature in Tunisian local goat kids during summer season. Proc. XI International Conference on Goats, Gran Canaria, Spain, 23-27/09/2012, p. 127.

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

Hamzaoui, S., Salama, A.A.K., Caja, G., Albanell, E., Flores, C., Such, X. 2012. Milk production losses in early lactating dairy goats under heat stress. J. Dairy Sci. 95 (2), 672–673.

Hassen, A., Theart, J.J.F., Van Niekerk, W.A., Adejoro, F.A., Gemeda, B.S. 2016. In vitro methane and gas production characteristics of Eragrostis trichopophora substrate supplemented with different browse foliage. Anim. Prod. Sci. 56, 634–640.

Hatfield, J., Boote, K., Fay, P., Hahn, L.C., Izaurralde, B.A., Kimball, T., Mader, J., Morgan, D., Ort, W., Polley, A., Thomson, W.D. 2008. Agriculture. In: The effects of climate change on agriculture, land resources, water resources, and biodiversity in the United States. A Report by the U.S. Climate Change Science Program and the Subcommittee on Global Change Research, Washington, DC, p 362.

Henke, A., Dickhoefer, U., Westreicher-Kristen, E., Knappstein, K., Molkentin, J., Hasler, M., Susenbeth, A. 2017. Effect of dietary Quebracho tannin extract on feed intake, digestibility, excretion of urinary purine derivatives and milk production in dairy cows. Arch. Anim. Nutr. 71, 37–53. https://doi.org/10.1080/1745039X.2016.1250541

Hill, R.W., Wyse, G.A., Anderson, M. 2012. Animal physiology. Sunderland, MA: Sinauer Associates, Inc.

Hirayama, T., Katoh, K., 2004. Effects of heat exposure and restricted feeding on behaviour, digestibility and growth hormone secretion in goats. Asian-australas. J. Anim. Sci. 17, 655–658. https://doi.org/10.5713/ajas.2004.655

Hoehn, A.N., Titgemeyer, E.C., Nagaraja, T.G., Drouillard, J.S., Miesner, M.D., Olson, K.C. 2018. Effects of high condensed-tannin substrate, prior dietary tannin exposure, antimicrobial inclusion, and animal species on fermentation parameters following a 48 h in vitro incubation. J. Anim. Sci. 96, 343–353. https://doi.org/10.1093/jas/skx018

Hooda, O.K., Upadhyay, R.C., 2014. Physiological responses, growth rate and blood metabolites under feed restriction and thermal exposure in kids. J. Stress Physiol. Biochem. 10, 214–227.

Howarth, R.W. 2015. Methane emissions and climatic warming risk from hydraulic fracturing and shale gas development; implication for policy. Energy Emiss. Control Technol. 45-53. https://doi.org/10.2147/EECT.S61539

Hristov, A., Oh, J., Firkins, J., Dijkstra, J., Kebreab, E., Waghorn, G., Makkar, H.P.S., Adesogan, A.T., Yang, W., Lee, C., Gerber, P.J., Henderson, B., Tricarico, J.M. 2013. SPECIAL TOPICS–Mitigation of methane and nitrous oxide emissions from animal operations: I. A review of enteric methane mitigation options. J. Anim. Sci. 91, 5045–5069. doi: 10.2527/jas.2013-6583

Hungate, R.E., Smith, W., Bauchop, T., Yu, I., Rabinowitz, J.C. 1970. Formate as an intermediate in the bovine rumen fermentation. J. Bacteriol. 102, 389–397. https://doi.org/10.1128/jb.102.2.389-397.1970

Hungate, R.E. 1967. Hydrogen as an intermediate in the rumen fermentation. Arch. Microbiol. 59, 158–164.

IPCC (International Panel on Climate Change). 2006. Revised IPCC Guidelines for National Greenhouse Gas Inventories. Chapter 10, Vol. 4: Agriculture, forestry and other land use. http://www.ipcc-nggip.iges.or.jp/public/2006gl/vol4.htm.

Jackson, P., Cockcroft, P. 2002. Clinical Examination of Farm Animals. Wiley-Blackwell, Osney Mead, Oxford, UK.

Jayanegara, A., Goel, G., Makkar, G.P.S., Becker, K. 2015. Divergence between purified hydrolysable and condensed tannin effects on methane emission, rumen fermentation and microbial population in vitro. Anim. Feed Sci. Technol. 209, 60–68. https://doi.org/10.1016/j.anifeedsci.2015.08.002

Jayanegara, A., Gustanti, R., Ridwanban, R., Widyastuti, Y. 2020. Fatty acid profiles of some insect oils and their effects onin vitrobovinerumen fermentation and methanogenesis. Ital. J. Anim. Sci. 19, 1310–1317. https://doi.org/10.1080/1828051X.2020.1841571

Joshi, B.C., McDowell, R.E., Sadhu, D.P. 1968. Effect of drugs on sweating rates in Hariana cattle. J. Dairy Sci. 51, 905–909. https://doi.org/10.3168/jds.S0022-0302(68)87101-2

Johnson, H.D. 1987. Bioclimates and Livestock. In Bioclimatology and the Adaptation of Livestock (ed. Johnson, H.D.), Elsevier Science Publishers, Amesterdam, pp. 3-16.

Johnson, K. A., and D. E. Johnson. 1995. Methane emissions from cattle. J. Anim. Sci. 73, 2483–2492. https://doi.org/10.2527/1995.7382483x

Kadim, I., Mahgoub, O., Al-Kindi, A., Al-Marzooqi, W., Al-Saqri, N.M. 2006. Effects of transportation at high ambient temperatures on physiological responses, carcass and meat quality characteristics of three breeds of omani goats. Meat Sci, 73(4), 626–634.

Kadzere, C. T., Murphy, M.R., Silanikove, N., Maltz, E. 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

Khan, M.S., Ghosh, P.K. 1989. Physiological responses of desert sheep and goats to grazing during summer and winter. Indian J. Anim. Sci. 59, 600–603.

Kliber, H.H. 1964. Environmental ohysiology and shelter engineering with special reference to domestic animals: LXVII. Thermal effects of various temperature-humidity combinations on Holstein cattle as measured by eight physiological responses. Agric. Exp. Station Res. Bull. 862. (University of Missouri, Columbia, MO).

Kyoto Protocol. 2009. United Nations Framework Convention on Climate Change. Accessed Nov. 26, 2009. http://unfccc.int/kyoto_protocol/items/2830.php

Knapp, J.R., Laur, G.L., Vadas, P.A., Weis, W.P., Tricarico, J.M. 2014. Invited review: enteric methane in dairy cattle production: quantifying the opportunities and impact of reducing emissions. J. Dairy Sci. 97, 3231–3261. https://doi.org/10.3168/jds.2013-7234

Lees, A.M., Lees, J.C., Sejian, V., Gaughan, J. 2017. Management Strategies to Reduce Heat Stress in Sheep. In: Sheep Production Adapting to Climate Change, Singapore Springer, pp. 359–380.

Li, Z., Deng, Q., Liu, Y., Yan, T., Li, F., Cao, Y., et al. 2018. Dynamics of methanogenesis, ruminal fermentation and fiber digestibility in ruminants following elimination of protozoa: a meta-analysis. J. Anim. Sci. Biotechnol. 9, 1–9. doi: 10.1186/s40104-018-0305-6

Lima, P.R., Apdini, T., Freire, A.S., Santana, A.S., Moura, L.M.L., Nascimento, J.C.S., Rodrigues, R.T.S., Dijkstra, J., Garcez Neto, A.F., Queiroz, M.A.A., Menezes., D.R. 2019. Dietary supplementation with tannin and soybean oil on intake, digestibility, feeding behavior, ruminal protozoa and methane emission in sheep.

Anim. Feed Sci. Technol. 249, 10–17. https://doi.org/10.1016/j.anifeedsci.2019.01.017

Liu, Y., Whitman, W.B. 2008. Metabolic, phylogenetic, and ecological diversity of the methanogenic archaea. Ann. N. Y. Acad. Sci. 1125, 171–189. https://doi.org/10.1196/annals.1419.019

Liu, Y., Xiao, Y., Ma, T., Diao, Q., Tu, Y. 2023. Candida tropicalis as a novel dietary additive to reduce methane emissions and nitrogen excretion in sheep. Environ. Sci. Pollut. Res. 30, 82661–82671 https://doi.org/10.1007/s11356-023-28245-x

Machmüller, A., Soliva, C. R., and Kreuzer, M. 2003. Methane-suppressing effect of myristic acid in sheep as affected by dietary calcium and forage proportion. Br. J. Nutr. 90, 529–540. doi: 10.1079/bjn200 3932

Madden, C.J., Morrison, S.F. 2019. Central nervous system circuits that control body temperature. Neurosci. Lett. 696, 225–232.

Malik, P., Kolte, A., Baruah, L., Saravanan, M., Bakshi, B., Bhatta, R. 2017. Enteric methane mitigation in sheep through leaves of selected tanniniferous tropical tree species. Livest. Sci. 200, 29–34. https://doi.org/10.1016/j.livsci.2017.04.001

Marai, I.F.M., Shalaby, T.H., Bahgat, L.B., Abdel-Hafez, M.A. 1997. Fattening of lambs on concentrates mixture diet alone without roughages or with addition of natural clay under subtropical conditions of Egypt. Growth performance and behaviour. In: Proceedings of International Conference on Animal Production & Health, Dokki, Cairo, Egypt.

Marai, I.F.M., Bahgat, L.B., Shalaby, T.H., Abdel-Hafez, M.A. 2000. Fattening performance, some behavioural traits and physiological reactions of male lambs fed concentrates mixture alone with or without natural clay, under hot summer of Egypt. Ann. Arid. Zone. 39, 449–460.

Marai, I.F., El Darawany, A.A., Fadiel, A., Abdel-Hafez, M.A.M. 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

Marino, R., Atzori, A.S., D'Andrea, M., Iovane, G., Trabalza-Marinucci, M., Rinaldi, L. 2016. Climate change: Production performance, health issues, greenhouse gas emissions and mitigation strategies in sheep and goat farming. Small Rumin. Res. 135, 50–59. https://doi.org/10.1016/j.smallrumres.2015.12.012.

Matthews, C., Crispie, F., Lewis, E., Reid, M., O'Toole, P.W., Cotter, P. 2019. The Rumen Microbiome: A Crucial Consideration When Optimising Milk and Meat Production and Nitrogen Utilisation Efficiency. Gut Microbes. 10, 115–132. https://doi.org/10.1080/19490976.2018.1505176

McDowell, R.E. 1972. Improvement of livestock production in warm climates. Freeman, San Francisco, pp 410–449

McManus, C., Paludo, G.R., louvandini, H., Gurgel, R., Sasaki, L.C.B., Paiva, S.R. 2009a. Heat tolerance in brazilian sheep: physiological and blood paóeters. Trop. Anim. Health Prod. 4, 95–101.

McManus, C., Prescott, E., Paludo, G.R., Bianchini, E., Louvandini, H., Mariante, A.S. 2009b. Heat tolerance in naturalized Brazilian cattle breeds. Livest. Sci. 120, 256–264. https://doi.org/10.1016/j.livsci.2008.07.014

Mili, B., Chutia, T. 2021. Adaptive Mechanisms of Goat to HeatStress. In Goat Science-Environment, Health and Economy (ed. Kukovics, S.), Intech Open, London, UK, pp. 1–16. DOI: 10.5772/intechopen.96874

Moate, P.J., Williams, S.R.O., Grainger, C., Hannah, M.C., Ponnampalam, E.N., Eckard, R.J. 2011. Influence of cold-pressed canola, brewers grains and hominy meal as dietary supplements suitable for reducing enteric methane emissions from lactating dairy cows. Anim. Feed Sci. Technol. 166–167, 254–264. https://doi.org/10.1016/j.anifeedsci.2011.04.069

Monty, D.E., Kelly, L.M., Rice, W.R. 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

Moorby, J.M., Fleming, H.R., Theobald, M.J., Fraser, M.D. 2015. Can live weight be used as a proxy for enteric methane emissions from pasture fed sheep? Sci. Rep. 5, 17915.

Morgavi, D.P., Jouany, J.P., Martin, C., 2008. Changes in methane emission and rumen fermentation parameters induced by refaunation in sheep. Aust. J. Exp. Agric. 48, 69–72. https://doi.org/10.1071/EA07236

Morgavi, D.P., Forano, E., Martin, C., Newbold, C.J. 2010. Microbial ecosystem and methanogenesis in ruminants. Animal. 4(7), 1024–1036.

Mota-Rojas, D., Titto, C.G., Orihuela, A., Martínez-Burnes, J., Gómez-Prado, J., Torres-Bernal, F., Flores-Padilla, K., Carvajal-de la Fuente, V., Wang, D. 2021. Physiological and Behavioral Mechanisms of Thermoregulation in Mammals. Animals. 11, 1733. https://doi.org/10.3390/ani11061733

Mueller-Harvey, I. 2006. Unravelling the conundrum of tannins in animal nutrition and health. J. Sci. Food Agric. 86, 2010–2037. https://doi.org/10.1002/jsfa.2577

Munin, A., Edwards-Lévy, F. 2011. Encapsulation of natural polyphenolic compounds; A review. Pharmaceutics. 3, 793–829. https://doi.org/10.3390/pharmaceutics3040793

Nardone, A., Lacetera, N., Ronchi, B, Bernabucci, U. 1992. Effetti dello stress termico sulla produzione di latte e sui consumi alimentari di vacche Frisone. Prod. Anim. 5, 1–15

Nardone, A., Ronchi, B., Valentini, A. 1991. Effects of solar radiation on water and food intake and weight gain in Sarda and Comisana female lambs. In: Animal Husbandry in Warm Climates, vol. 55. EAAP Publication, pp. 149–150.

National Research Council (US) Subcommittee on Environmental Stress. 1981. Effect of Environment on Nutrient Requirements of Domestic Animals. Washington (DC): National Academies Press (US).

Naumann, H.D., Armstrong, S.A., Lambert, B.D., Muir, J.P., Tedeschi, L.O., Kothmann, M.M. 2014. Effect of molecular weight and concentration of legume condensed tannins on in vitro larval migration inhibition of Haemonchus contortus. Vet. Parasitol. 199, 93–98. https://doi.org/10.1016/j.vetpar.2013.09.025

Newbold, C.J., de la Fuente, G., Belanche, A., Ramos-Morales, E., McEwan, N.R. 2015. The Role of Ciliate Protozoa in the Rumen. Front. Microbiol. 6, 1313. https://doi.org/10.3389/fmicb.2015.01313

Newbold, C.J., Ramos-Morales, E. 2020. Review: Ruminal Microbiome and Microbial Metabolome: Effects of Diet and Ruminant Host. Animal. 14(1), s78–s86.

Nolan, J., Hegarty, R., Hegarty, J., Godwin, I., Woodgate, R. 2010. Effects of dietary nitrate on fermentation, methane production and digesta kinetics in sheep. Anim Prod Sci. 50. 801–6. https://doi.org/10.1071/AN09211

NRC. 2007. Nutrient Requirements of Small Ruminants, Sheep, Goats, Cervids, and New World Camelids. National Academy Press, Washington, DC.)

Okoruwa, M.I. 2014. Effect of heat stress on thermoregulatory, live bodyweight and physiological responses of dwarf goats in southern Nigeria. Eur. Sci. J. 10, 255–264.

Pathak, A.K., Dutta, N., Pattanaik, A.K., Chaturvedi, V.B., Sharma, K. 2017. Effect of condensed tannins from Ficus infectoria and Psidium guajava leaf meal mixture on nutrient metabolism, methane emission and performance of lambs. Ruminant Nutrition and Forage Utilization. Asian-Australas. J. Anim. Sci. 30(12), 1702–1710. 10.5713/ajas.17.0060

Patra, A.K., Saxena, J. 2011. Exploitation of dietary tannins to improve rumen metabolism and ruminant nutrition. J. Sci. Food Agric. 91, 24–37. https://doi.org/10.1002/jsfa.4152

Patra, AK. 2012. Enteric methane mitigation technologies for ruminant livestock: a synthesis of current research and future directions. Environ. Monitor. Assess. 184, 1929–1952.

Peana, I., Fois, G., Cannas, A. 2007a. Effects of heat stress and diet on milk production and feed and energy intake of Sarda ewes. Ital. J. Anim. Sci. 6(1), 577–579. https://doi.org/10.4081/ijas.2007.1s.577

Peana, I., Dimauro C., Carta M., Gaspa M., Fois G., Cannas, A. 2007b. Effects of heat stress on milk yield in Sardinian dairy sheep farms. Proceedings of this Symposium.

Pennisi, P., Costa, A., Biondi, L., Avondo, M., Piccione, G. 2004. Influence of the fleece on thermal homeostasis and on body condition in Comisana ewe lambs. Anim. Res. 53, 13–19. https://doi.org/10.1051/animres:2003047

Perdomo, M.C., Marsola, R.S., Favoreto, M.G., Adesogan, A., Staples, C.R., Santos, J.E.P. 2019. Effects of feeding live yeast at 2 dosages on performance and feeding behavior of dairy cows under heat stress. J. Dairy Sci. 103, 325–339. https://doi.org/10.3168/jds.2019-17303

Phulia, S.K., Upadhyay, R.C., Jindal, S.K., Misra, R.P. 2010. Alteration in surface body temperature and physiological responses in Sirohi goats during daytime in summer season. Indian J. Anim. Sci. 80, 340–342.

Piccione, G., Lutri, L., Casella, S., Ferrantelli, V., Pennisi, P. 2008. Effect of shearing and environmental conditions on physiological mechanisms in ewes. J. Environ. Biol. 29, 877–880. http: //www.geocities.com/j_environ_biol/

Piñeiro-Vázquez, A.T., Canul-Solis, J.R., Jiménez-Ferrer, G.O., Alayón-Gamboa, J.A., Chay-Canul, A.J., Ayala-Burgos, A.J., Aguilar-Pérez, C.F., Ku-Vera, J.C. 2018. Effect of condensed tannins from Leucaena leucocephala on rumen fermentation, methane production and population of rumen protozoa in heifers fed low-quality forage. Asian-Australas J. Anim. Sci. 31(11), 1738–1746. 10.5713/ajas.17.0192

Popoola, M.A., Bolarinwa, M.O., Yahaya, M.O., Adebisi, G.L., Saka, A.A. 2014. Thermal comfort effects on physiological adaptations and growth performance of West African dwarf goats raised in Nigeria. Eur. Sci. J. 3, 275–382.

Ramin, M., Huhtanen, P. 2013. Development of equations for predicting methane emissions from ruinants. J. Dairy Sci. 96, 2476–2493. https://doi.org/10.3168/jds.2012-6095

Rashamol, V.P., Sejian, V., Bagath, M., Krishnan, G., Archana, P.R., Bhatta, R. 2018. Physiological adaptability of livestock to heat Stress: an updated review. J. Anim. Behav. Biometeorol. 6, 62–71. http://dx.doi.org/10.31893/2318-1265jabb.v6n3p62-71

Rashwan Ali, A., Ibrahim Fayez, M., & Marai, I. F. M. 2004. Rabbits behavioural response to climatic and managerial conditions-are view. Arch Tierzucht Dummerstorf. 47(5), 469–482.

Reisinger, A., Clark, H., Cowie, A., Emmet-Booth, J., Gonzalez Fischer, C., Herrero, M., et al. 2021. How necessary and feasible are reductions of methane emissions from livestock to support stringent temperature goals. Philos. Trans. Royal. Soc. A. 379, 20200452. https://doi.org/10.1098/rsta.2020.0452

Rhoads, R.P., Baumgard, L.H., Suagee, J.K. 2013. 2011 AND 2012 EARLY CAREERS ACHIEVEMENT AWARDS: Metabolic priorities during heat stress with an emphasis on skeletal muscle. J. Anim. Sci. 91(6), 2492–2503. https://doi.org/10.2527/jas.2012-6120

Ribeiro, N.L., Costa, R.G., Filho, E.C.P., Ribeiro, M.N., Bozzi, R. 2018. Effects of the dry and the rainy season on endocrine and physiologic profiles of goats in the Brazilian semi-arid region. Ital. J. Anim. Sci. 17(2), 454–461. https://doi.org/10.1080/1828051X.2017.1393320

Roskopf, P.M., Tieri, M.P., Cuatrin, A., Cucchi, M.E.C., Gere, J.I., Salado, E.E. 2023. Performance of Dairy Cows Supplemented with By-Pass Fat under Heat Stress Conditions. Open J. Anim. Sci. 13, 82–97. 10.4236/ojas.2023.131006

Sallam, S.M.A., Nasser, M.E.A., Araujo, R.C., Abdalla, A.L. 2009. Methane emission in vivo by sheep consuming diet with different levels of eucalyptus essential oil 210 211 Proc. FAO/IAEA Int. Symp on sustainable improvement of animal production and health Vienna, Austria.

Salama, A.A.K., Hamzaoui, S., Caja, G. 2012. Responses of dairy goats to heat stress and strategies to alleviate its effects. Proc. XI International Conference on Goats, Gran Canaria, Spain. 23–27.09.2012, p. 15.

Salama, A.A.K, Caja, G., Hamzaoui, S., Badaoui, B., Castro-Costa, A., Façanha, D.A.E., Guilhermino, M.M., Bozzi, R. 2014. Different levels of response to heat stress in dairy goats. Small Rumin. Res. 121, 73–279. https://doi.org/10.1016/j.smallrumres.2013.11.021

Sanjorjo, R.A., Tseten, T., Kang, M.K., Kwon, M., Kim, S.W. 2023. In Pursuit of Understanding the Rumen Microbiome. Fermentation. 9, 114. https://doi.org/10.3390/fermentation9020114

Sano, H., Ambo, K., Tsuda, T. 1985. Blood glucose kinetics in whole body and mammary gland of lactating goats exposed to heat stress. J. Dairy Sci. 68, 2557–2564. https://doi.org/10.3168/jds.S0022-0302(85)81137-1

Sarangi, S. 2018. Adaptability of goats to heat stress: A review. J. Pharm. Innov. 7, 1114–1126

Schutz, K.E., Roger, A.R., Cox, N.R., Webster, J.R. and Tucker, C.B. 2011. Dairy cattle prefer shade over sprinkler effects on behaviour and physiological. J. Dairy Sci. 94, 273–283. https://doi.org/10.3168/jds.2010-3608

Schneider, P.L., Beede, D, K., Wilcox, C. J., Collier, R.J. 1984. Influence of Dietary Sodium and Potassium Bicarbonate and Total Potassium on Heat-Stressed Lactating Dairy Cows. J. Dairy- Sci. 67, 2546–2553. https://doi.org/10.3168/jds.S0022-0302(84)81611-2

Sejian, V., Maurya, V.P., Naqvi, S.M.K. 2012. Effect of walking stress on growth, physiological adaptability and endocrine responses in Malpura ewes under semi-arid tropical environment. Int. J. Biometeorol. 56, 243–252.

Sejian, V., Bhatta, R., Gaughan, J., Malik, P.K., Naqvi, S.M.K., Lal, R. 2017. Sheep production adapting to climate change. Singapore Springer.

Sejian, V., Bhatta, R., Gaughan, J.B., Dunshea, F.R., Lacetera, N. 2018. Review: Adaptation of animals to heat stress. Animal. 12, 431–444

Sevi, A., Annicchiarico, G., Albenzio, M., Taibi, L., Muscio, A., Dell'Aquila, S. 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

Sevi, A., Albenzio, M., Annicchiarico, G., Caroprese, M., Marino, R., Taibi, L. 2002. Effects of ventilation regimen on the welfare and performance of lactating ewes in summer. J. Anim. Sci. 80, 2349–2361. https://doi.org/10.1093/ansci/80.9.2349

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

Shalaby, T.H. 1985. Performance and adaptation of local sheep to varied environmental and managerial conditions. Ph.D. thesis. Faculty of Agriculture, Cairo University, Cairo, Egypt.

Shan, Q., Ma, F.T., Jin, Y.H., Gao, D., Li, H.Y., Sun, P. 2020. Chromium yeast alleviates heat stress by improving antioxidant and immune function in Holstein mid-lactation dairy cows. Anim. Feed Sci. Technol. 269, 114635. https://doi.org/10.1016/j.anifeedsci.2020.114635

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., Leitner, G., Merin, U., Prosser, C.G. 2010. Recent advances in exploiting goat’s milk: quality, safety and production aspects. Small Rumin. Res. 89, 110–124. https://doi.org/10.1016/j.smallrumres.2009.12.033

Slimen, I.B, Chniter, M., Najar, T., Ghram, A. 2019. Meta-analysis of some physiologic, metabolic and oxidative responses of sheep exposed to environmental heat stress. Livest. Sci. 229, 179-187. https://doi.org/10.1016/j.livsci.2019.09.026

Smith, B., McNabb, D., Smithers, J. 1996. Agricultural adaptation to climatic variation. Climate Change. 43, 7–29.

Smith, C.J., Johnson, J.M. 2016. Responses to hyperthermia. Optimizing heat dissipation by convection and evaporation: Neural control of skin blood flow and sweating in humans. Auton. Neurosci. 196, 25–36.

Soltan, Y.A., Natel., A.S., Araujo, R.C., Morsy, A.S., Abdalla, A.L. 2018. Progressive adaptation of sheep to a microencapsulated blend of essential oils: Ruminal fermentation, methane emission, nutrient digestibility, and microbial protein synthesis. Anim. Feed Sci. Technol. 237, 8–18. https://doi.org/10.1016/j.anifeedsci.2018.01.004

Srikandakumar, A., Johnson, E.H., Mahgoub, O. 2003. Effect of heat stress on respiratory rate, rectal temperature and blood chemistry in Omani and Australian Merino sheep. Small Rum. Res. 49, 193–198. https://doi.org/10.1016/S0921-4488(03)00097-X

Steinfeld, H., Gerber, P., Wassenaar, T.D., Castel, V. & de Haan, C. 2006. Livestock's long shadow: environmental issues and options. Food Agric. Organiz. United Nations, Rome.

Tan, C., Ramírez-Restrepo, C. A., Shah, A. M., Hu, R., Bell, M., Wang, Z., et al. 2020. The community structure and microbial linkage of rumen protozoa and methanogens in response to the addition of tea seed saponins in the diet of beef cattle. J. Anim. Sci. Biotechnol. 11, 80. doi: 10.1186/s40104-020-00 491-w

Tavendale, M.M.H., Meagher, L.L.P., Pacheco, D., Walker, N., Attwood, G.T., Sivakumaran, S. 2005. Methane production from in vitro rumen incubations with Lotus pedunculatus and Medicago sativa, and effects of extractable condensed tannin fractions on methanogenesis. Anim. Feed Sci. Technol. 123, 403–419. https://doi.org/10.1016/j.anifeedsci.2005.04.037

Thirumalaisamy, G., Malik, P. K., Kolte, A. P., Trivedi, S., Dhali, A., and Bhatta, R. 2020. Effect of silkworm (Bombyx mori) pupae oil supplementation on enteric methane emission and methanogens diversity in sheep. Anim. Biotechnol. 33, 128–140. doi: 10.1080/10495398.2020.1781147

Thirumalaisamy, G., Malik, P.K., Trivedi, S., Kolte, A.P., Bhatta, R. 2022. Effect of Long-Term Supplementation With Silkworm Pupae Oil on the Methane Yield, Ruminal Protozoa, and Archaea Community in Sheep. Front. Microbiol. 13, 780073. https://doi.org/10.3389/fmicb.2022.780073

Thompson, G.E. 1985. Respiratory system. In Stress Physiology in Livestock (ed. Young, M.K.), CRC Press, Inc., Boca Raton, Horida, USA, pp. 155–162.

Thwaites, C.J. 1985. Physiological responses and productivity in sheep. In Stress Physiology in Livestock (es. Yousef, M.K.), CRC Press, Inc., Boca Raton, Florida, USA, pp. 25–39.

Tokura, M., Ushida, K., Miyazaki, K., Kojima, Y. 1997. Methanogens associated with rumen ciliates. FEMS Microbiol. Ecol. 22, 137–143. https://doi.org/10.1111/j.1574-6941.1997.tb00365.x

Torres, R.N.S., Coelho, L.M., Ghedini, C.P., Neto, O.R.M., Chardulo, L.A.L., Torrecilhas, J.A., Valença, R.L., Baldassini, W.A., Almeida, M.T.C. 2023. Potential of nutritional strategies to reduce enteric methane emission in feedlot sheep: A meta-analysis and multivariate analysis. Small Rum. Res. 220, 106919. https://doi.org/10.1016/j.smallrumres.2023.106919

Tüfekci, M. 2023. The effects of heat stress on some parameters in sheep and goats. ISPEC 11th International conference on agriculture, animal sciences and rural development. 03-05 march 2023.

United Nations Environment Programme and Climate and Clean Air Coalition. Global methane assessment: benefits and costs of mitigating methane emis sions. Nairobi: United Nations Environment Programme. 2021.

United Nations Framework Convention on Climate Change. 2015. UN Climate Change Newsroom. Historic Paris agreement on climate change. 195 nations set path to keep temperature rise well below 2 degrees Celsius. http://newsroom.unfccc.int/unfccc-newsroom/finale-cop21/.

Van Zijderveld, S.M., Gerrits, W.J.J., Apajalahti, J.A., Newbold, J.R., Dijkstra, J., Leng, R.A., Perdok, H.B. 2010. Nitrate and sulfate: Effective alternative hydrogen sinks for mitigation of ruminal methane production in sheep. J. Dairy Sci. 93, 5856–5866. https://doi.org/10.3168/jds.2010-3281

Vargas, J. E., Andrés, S., López-Ferreras, L., Snelling, T. J., Yáñez-Ruíz, D. R., García-Estrada, C., et al. 2020. Dietary supplemental plant oils reduce methanogenesis from anaerobic microbial fermentation in the rumen. Sci. Rep. 10, 1613. doi: 10.1038/s41598-020-58401-z

Vieira, R.A., Peripolli, V. and McManus, C. 2023. Path analysis and logistic regression for heat tolerance in sheep, Small Rumin. Res. 229, 107125 https://doi.org/10.1016/j.smallrumres.2023.107125.

Waghorn, G. 2008. Beneficial and detrimental effects of dietary condensed tannins for sustainable sheep and goat production-Progress and challenges. Anim. Feed Sci. Technol. 147, 116–139. https://doi.org/10.1016/j.anifeedsci.2007.09.013

West, J.W. 1999. Nutritional strategies for managing the heat stressed dairy cow. J. Anim. Sci. 77, 21–35. https://doi.org/10.2527/1997.77suppl_221x

Wildridge, A.M., Thomson, P.C., Garcia, S.C., John, A.J., Jongman, E.C., Clark, C.E.F., Kerrisk, K.L. 2018. Short communication: The effect of temperature-humidity index on milk yield and milking frequency of dairy cows in pasture-based automatic milking systems. J. Dairy Sci. 101, 4479–4482. https://doi.org/10.3168/jds.2017-13867

Williams, A.G., Coleman, G.S. 1992. The Rumen Protozoa. New York, NY: Springer-Verlag

Wood, T.A., Wallace, R.J., Rowe, A., Price, J., Yanez-Ruiz, D.R., Murray, P., Newbold, C.J. 2009. Encapsulated fumaric acid as a feed ingredient to decrease ruminal methane emissions. Anim. Feed Sci. Technol. 152, 62–71. https://doi.org/10.1016/j.anifeedsci.2009.03.006

Wu, Y., Nieuwenhoff, M., Huygen, F., van der Helm, F., Niehof, S., Schouten, A. 2017. Characterizing human skin blood flow regulation in response to different local skin temperature perturbations. Microvasc. Res. 111, 96–102.

Wuebbles, D.J., Hayhoe, K. 2002. Atmospheric methane and global change. Earth Sci. Rev. 57:117–210. Zimbelman, R.B., Baumgard, L.H., Collier, R.J., 2010. Effects of encapsulated niacin on evaporative heat loss and body temperature in moderately heat-stressed lactating Holstein cows. J. Dairy Sci. 93, 2387–2394. https://doi.org/10.3168/jds.2009-2557

Yanagita, K., Kamagata, Y., Kawaharasaki, M., Suzuki, T., Nakamura, Y., and Minato, H. 2000. Phylogenetic analysis of methanogens in sheep rumen ecosystem and detection of Methanomicrobium mobile by fluorescence in situ hybridization. Biosci. Biotechnol. Biochem. 64, 1737–1742. https://doi.org/10.1271/bbb.64.1737

Yuan, Z.P., Zhang, C.M., Zhou, L., Zou, C.X., Guo, Y.Q., Li, W.T., Liu, J.X., Wu, Y.M. 2007. Inhibition of methanogenesis by tea saponin and tea saponin plus disodium fumarate in sheep. J. Anim. Feed Sci. 16(2), 560–565. DOI: https://doi.org/10.22358/jafs/74607/2007

Zhao, Y., Nan, X., Yang, L., Zheng, S., Jiang, L., Xiong, B. 2020. A REVIEW OF Wnteric Methane Emission Measurement Techniques in Ruminants. Animals. 10(6), 1004. https://doi.org/10.3390/ani10061004

Zhao, Y., Zhao, G. 2022. Decreasing ruminal methane production through enhancing the sulfate reduction pathway. Anim. Nutr. 9, 320–326. https://doi.org/10.1016/j.aninu.2022.01.006

Zimbelman, R.B., Collier, R.J., Bilby, T.R. 2014. Effects of utilizing rumen protected niacin on core body temperature as well as milk production and composition in lactating dairy cows during heat stress. Anim. Feed Sci. Technol. 180 (1-4), 26–33. https://doi.org/10.1016/j.anifeedsci.2013.01.005

OBJECTIVES OF THE THESIS

Objective 1 (General objective). The general objective of the experimental work described in this dissertation was to explore the response of Sarda sheep to heat stress and to collect data to be further use for modeling the sheep response to heat stress. The work has to be considered preliminary for the understanding of the complex mechanism of animal response to heat exchange, accumulation and productive response. It includes the study of the literature and the planning of the experimental work and the summarizing of all the resuls in a final chapter of general conclusions.

Objective 2. Ths objective was addressed with the first experimental trial presented in the dissertation, it aimed to define the response to daily THI and temperature variation in sheep housed indoor without forced cooling systems and measured at different production level. This work aims to indirectly quantify the metabolic effort of the animal as response to warm climate inside the barn using indirect markers of heat accumulation measurable on field.

Objective 3. The objective was addressed with the third experimental trial presented in the dissertation, it aimed to compare markers of heat stress in sheep from summer to winter and comparing them with data obtained from a group of animals in the previous winter during a similar trial conducted in the same barn. It should help to understand the difference in metabolic effort between no stress period and summer time.

Objective 4. The objective was addressed with the fourth experimental trial presented in the dissertation, it aimed to quantify the effects of heat stress on Sarda dairy sheep performances in lactation and fed different forages and relate it with the main indicators of heat stress (respiration rate and rectal temperature).

Objective 5. The objective was addressed with the fifth experimental trial presented in this dissertation, it aimed to investigate the correlations among body measures and surface and volume of the sheep to develop a geometric model of the sheep surface and volume. It also included a prelimirary system dynamics modeling approach to predict heat production and dissipation in dairy sheep. A conceptual qualitative model using causal loop diagramming was carried out in this work.

Objectve 6. The objective was addressed with the sisth experimental trial presented in this dissertation, it aimed to study the relationship among methane emission and respiration rate on heat stress conditions during an experiment carried out in ventilated hoods where sheep were housed for direct measures of methane emissions during summer 2022. During this trial several measures of heat stress indicators were collected.

CHAPTER 2

Field measures for quantification of the response to summer air temperature in Sarda sheep housed indoor

2.1. Abstract

Sheep are considered one of the most favoured species to adapt to climate change. Nevertheless, they suffer heat stress more intensively thant cattle due to their high metabolic effort per kg of BW and the wool coat. Biological markers of heat stress need to be found at field level and to develop quantitative approach to increase of the energy requirements due to heat stress. The study aimed to quantify the response to daily THI and temperature variation in Sarda sheep at different production level and housed indoor without forced cooling systems by using indirect markers of heat accumulation measurable on field. Fifteen Sarda dairy ewes were divided in three groups homogenous for Body Weight (BW; 48.2±3.57 kg) and different for milk yield (MY): High MY, Low MY and Dry with 1900, 1200, 0 g/d of milk per ewe (n=5), respectively. Air temperature and relative humidity were recorded inside the barn and the Temperature humidity index was calculated for 60 days in summer 2020. Five times per day at 6.00, 9.00, 12.00, 15.00 and 18.00 hours,nine physiological variables were measured on the individual animals: the respiratory rate (RR), pulse heart rate (HR), blood oxygen saturation (OX), mammary, ear and skin temperature (UT, ET, ST respectively) and rectal and vagina temperature (RT and VT). With increasing of THI classes a significant increase was observed for all animal measures ($P< 0.001$). In particular, in RR, HR showed the broadest range of variation with temperature increase from THI <70 to >80 the observed percentage increase in measured variables was: 65% for RR, 49% for HR, 1% for RT, 1% for VT, 2% ET and 1% for UT. The basal value for RR, HR, and RT was on average 85.23 and act/min, 58.64 beat/min and 39.36 °C with THI <70, respectively. When all data were plotted, RR was highly associated with air temperature ($R^2 = 0.58$) than THI ($R^2 = 0.26$). Lactating animals showed high stress in comparison to dry groups even with medium-low milk production level. In conclusion, respiration rate is the most valuable marker for heat stress to be used at field level for housed sheep.

Keywords: respiration rate, THI, indoor, lactating sheep

2.2. Introduction

Sheep are considered one of the most favoured species to adapt to climate change (Sejian et al., 2017). Temperature, humidity, vegetation, wool cover influences the adaptation of sheep breeds to different locations, climates, resistance and susceptibility to various diseases. Someone sheep breeds can tolerate a wide range of climate and convert poor-quality forage into quality animal protein. These characteristics favour their rearing under extensive system among poor rural people in harsh climate (Shinde and Sejian, 2013). Mediterranean summer conditions are characterized by high variations of daily temperatures, which often cause heat stress in dairy sheep (Sevi and Caroprese, 2012; Peana et al., 2017). Heat stress refers to low capacity of the animal to dissipate excesses of heat and to maintain thermal homeostasis as consequence of high endogenous heat production due to nutrition level and of high environmental temperatures (Sejian et al., 2017). Several studies estimated the animal response to environmental condition change. They showed that the increase of respiratory rate, rectal temperature, hearth rate and skin temperature aim to enhance heat losses (conduction, convection, radiation; water diffusion from the skin) by increasing blood flow from the core to the surface and facilitate heat dissipation (Sejian et al., 2017). This response to heat stress increases energetic maintenance requirements thus the quantification of the metabolic efforts for thermoregulation are useful to adjust feeding requirements of sheep. In this sense physiological measures can be target as biomarkers of energy expenditure to predict changes in energy metabolism and improve feeding models. It is important to target the measures that can be obtained at field level with the perspective to develop new algorithms able to quantify the variation in animal requirements due to heat stress. One of the most important physiological parameters, used as markers of heat stress, is the respiratory rate (Rashamol et al., 2018). Under neutral environmental temperature (12 °C) sheep loses about 20% of total body heat via respiratory tracts (Marai, 2007). Respiration rates for adult sheep ranges between 20-30 breaths/min (Jakson and Cockcroft, 2002). Marai et al. (2007) observes that respiratory rates during summer increase through the day.

Another parameter, important to measure the physiological status is the rectal temperature (Rashamol et al., 2018). With this value it's possible to understand the measure of animal's core body temperature and an increasement represent the failure of thermoregulatory mechanism with respiration and sweat. In sheep, the normal rectal temperatures range between 38.3-39.9 °C. Whit increases of the air temperature from thermoneutral conditions (i.e.: 18 °C) to heat stress conditions (i.e.: 35 °C), the rectal temperature has been observed to markedly increased in sheep in many studies cited by Marai et al., (2007). It has to be noticed that there is a risk of death with a rectal temperature above 42 °C (Thwaites, 1985). Vaginal temperature also reflects body temperature, and many studies demonstrated a high correlation between the two measures, with negligible differences (Baida et al., 2022). Pulse rate is a physiological parameter, reliable indicator of heat stress in livestock since cardiorespiratory system is influenced by season, day timings, ambient temperature, humidity, exercise (Rashmol et al., 2018). Normal range resting pulse rate (beats/min) is 70-90 in sheep (Jakson and Cockcroft, 2002), but it changes with breeds and latitude. The rate increases in case of exposure to high ambient temperatures (Aboul-Naga, 1987) causing an acceleration of the blood flow in such a way as to make a greater dispersion of heat possible. With an increase of pulse rate, body lose sensibly (conduction, convection, radiation) and insensible heat (water diffusion from the skin) by increasing blood flow from the core to the surface (Rashamol et al., 2018). It reflects the homeostasis of circulation along with the general metabolic status (Marai et al., 2007). Skin temperature also depends to the exposition level to sun rays of the different body part and by his blood flow and it is influenced by the increase of solar radiation and air temperature, as the time of the day and the season (Fahmy, 1994). When environmental temperature is greater than skin temperature, the temperature gradient between the body surface and the ambience decreases reduces heat dissipation by evaporation (Mili and Chutia, 2021). Sweating, as panting, are two automatic primary responses of an animal under heat stress (Gaughan and Cawdell-Smith, 2015) even if in sheep sweating is much less important than respiratory evaporation because of the presence of a wool coat.

The study aimed to quantify the response to daily THI and temperature variation in Sarda sheep at different production level and housed indoor without forced cooling systems by using indirect markers of heat accumulation measurable on field.

2.3. Materials and methods

The experiment was conducted in the experimental farm of the Department of Agriculture of University of Sassari located in the north-west of Sardinia (Sassari, Italy; 40°48’41.4” N 8°17’50.7” E). All procedures involving animals were fully in compliance with the European Community (86/609) and Italian regulations (DPR 27/1/1992, Animal Protection Regulations of 124 Italy) on animal welfare and experimentation. Fifteen Sarda dairy ewes were divided in three groups homogenous for Body Weight (BW; 48.2±3.57 kg) and different for milk yield (MY): High MY, Low MY and Dry with 1900, 1200, 0 g/d of milk per ewe (n=5), respectively. Ewes were housed in 3 separate pens of 15 m^2. The barn was not equipped with cooling systems. Animals were fed two times per day with alfalfa and ryegrass hay (about 55% of forage for lactating and 75% for dry) and a mix of concentrates composed by soybean meal (about 10% of DMI) for the only lactating groups and by beet pulp (35% for lactating and 25% for dry groups). The animals were fed the same diet offered with a different amount in order to cover the nutritional requirements according to their milk yield and body weight, calculated with the software small Ruminant nutrition system (www.nutritionmodels.edu). Physiological response to environmental air temperature and THI (Kliber, 1964) were monitored one day per week for 60 days between July and September 2020. Whether variables were measured inside the barn using a whether station (PCE Italia s.r.l., PCE-FWS 20N, Lucca, Italy) with data recording every minute. Dowloaded data from the whether station were further elaborated. Measures on the animals were recorded 5 times per day at 6.00, 9.00, 12.00, 15.00 and 18.00 hours. Nine physiological variables were measured on the individual animals of each group: the respiratory rate (RR; measured with manual counts within recorded videos of 1 minute); pulse heart rate (HR; beat per minute using digital pulse oximeter Pic Solution®) and blood oxygen saturation (OX; using the same digital pulse oximeter Pic Solution®); mammary, ear and skin temperature (UT, ET, ST respectively; infrared digital thermometer Pic Solution®); rectal and vagina temperature (RT and VT; digital thermometers Pic Solution®); milk yield (MY) from the milking machine of the experimental barn (Afimilk®; at 07:00 and 19:00 hours daily). Statistical analyses were performed with the software SAS (9.0) with techniques of descriptive statistics and testing a mixed model for repeated measurements considering as dependent variables the above mentioned animal measures; as fixed effects four classes of THI (<70; 70-75; 76-80 and >80) and of air temperature in °C (<23; 23-25; 26-28, >28) and milk production (or group); as random effects hours, day and animal.

2.4. Results

With increasing of THI classes, a statistically significant increase in RR, HR and RT was observed ($P<0.0001$). The basal value for THI <70 was on average 85.23 and act/min, 58.64 beat/min and 39.36 °C for RR and RT, respectively. Similarly, was observed an increase in VT ($P<0.001$), ET ($P<0.001$) and UT ($P<0.05$) with minimum average values for THI <70 of 39.43 °C, 37.13 °C and 38.3 °C for VT, ET and UT, respectively (Table 1). Furthermore, MY also affected significantly the same variables ($P<0.0001$ for RT and VT; $P<0.001$ for RR; $P<0.05$ for UT). It has to be noticed that from THI <70 to >80 the observed percentage increase in measured variables was: 65% for RR, 49% for HR, 1% for RT, 1% for VT, 2% ET and 1% for UT.

Similarly, the air temperature class effect was tested to study the same relationships. It was possible to observe how the increase of AT caused a consequent statistically significant increase of RR, HR, RT, VT ET and UT parameters ($P<0.0001$ for the first two).

Milk production level significantly affected RT and VT ($P<0.0001$), RR ($P<0.01$) and UT ($P<0.05$). Figure 1 shows the regression between and breaths per minute vs. internal air temperature. Here, as the temperature increases, the frequency of respiratory acts increases proportionally. It explained the 58% of the daytime variability in the summer period considered. When all data were used the relationship can be resumed by the equations:

RR, acts/min = 8.0478 * AT (° C) - 105.23; $R^2 = 0.58$; $P < 0.01$ (Figure 1)

RR, acts/min = 4.52 * THI (°F) - 209.66; $R^2 = 0.26$; $P < 0.01$.

where RR is respiratory rate in breaths/min, THI is Temperature humidity index in °F and AT air temperature in °C.

It has to be noticed that RT and VT, related to internal body core temperature and heat accumulation, has been observed a strong relationship (R^2=0.94, P<0.001; Figure 2).

An hourly variation in physiological parameters among groups was observed (Figure 3). The trend of the respiratory rate (reported to the THI) indicated that from the coldest hour of the day (6:00; AT=23.3 °C and RH=75.8%), to the hottest hours (12:00 and 15:00; AT=27.4-27.8 °C and RH=64.7-65.6 %, respectively) animals suffer a continuous increase until the afternoon hours and a decrease with the stabilization of temperatures in the evening (18:00; AT=27.6 °C and RH=69%). It is also evident that in the early hours of the day all the group of animals have very similar responses. Otherwise with the aggravation of environmental conditions the lactating groups suffer greater heat stress than dry sheep, settling in a much higher range of the RR, RT, ET, ST and UT (P<0.05; figure from 3a to 3e). For RT, VT and ET is registered a continuous increase of the values going from the morning hours of the day to the hottest hours in which the difference between the lactating and dry groups is evident at each time. On the contrary for UT, as for RR, all the groups showed similar level in the morning but increasing with different patterns over the day (Figure 1). ST showed an opposite trend; and about OX dry group maintained a constant higher blood oxygen saturation than the two lactating groups (4.19 ± 1.8 SPO_2) (Figure 3f).

The milk production significantly decreased passing from low stress (low THI) to a heat stress condition (high THI) (P<0.001; Figure 4). The THI explained in this experiment more than 50% of the milk yield variability (Figure 4). A similar decrease of milk was observed in groups equal to 50 g/d and 30 g/d of milk yield reduction per each increase both of THI and a loss of production equal to -2.7% and -2.6%, respectively for the High and low MY group.

2.5. Discussions

Respiratory rate and heart rate appear to be the animal variables more associated with environmental conditions within the barn. It is not possible to adfirm that RR and HR are biologically the most important varaibles reflecting the heat accumulation in animal body but from a proxy point of view the broad range of their variability allows to easily measure those markers at field level and have an indication of animal response to barn environment.

Comparing this work results with the informations found in literature, it is possible to confirm that these variables are implied in the most important animals' physiological responses facing heat stress. Silanikove (2000), observed that the RR is strongly influenced by environmental conditions, and Slimen (2019), Aboul-Naga (1987) and Alexiev et al. (2004) observed an increase of RR on exposure to environmental high temperatures, also with an acceleration of heart rate in the hottest hours and with an increase of RT trend (Mascarenhas et al., 2023). The daily variation indicated by (Marai et al., 2007; Shalaby, 1985) about RR and RT is also similar to what observed in this work. The importance as markers of heat stress of physiological parameter such as HR, ST is confirmed also in the review of Sejian et al. (2017). In the specific case of HR, the value observed in no stress conditions established by Jackson and Cockcroft (2002) was 70-90 beats/min, higher than the values observed in the present work (60 beaths/min). Contrarly in Slimen (2019) study, HR trends in a opposite direction, with higher values at THI<72 (98.81) and low values at THI>77 (88.55) explained by different physiological stages of animals. Both in sheep (Hamadeh et al., 2006) and in cows (Turner et al., 2010), numerically higher RT and VT values were observed in lactating vs dry groups suggesting that the metabolic rate varies with physiological stages. It was related also to a higher heat dissipation demand from peripherical areas like ears

(Johnson 1987). The high correlation between RT and VT found in the presented work was closer to that obtained by George et al., (2014; R=88, P<0.001), but much higher than that observed by Baida et al., (2022; R=62.0, P<0.0001). A numerically higher level of oxygen in the blood recorded in dry animals compared to lactating animals, could be probably related to the difference between lactation and pregnancy status and differences in rquirements and metabolic demand joined with heat stress conditions (El-Sherif and Assad, 2001). The strong influence of environmental conditions observed in this study on milk yield are in line with those observed by Peana et al. (2007a) and Finocchiaro et al., (2005). The production loss observed in this work is also close to the 3% observed in late lactation goats by Hamzaoui et al. (2012).

2.6. Conclusions

The results obtained in the presented work indicate that heat stress causes an increase in the animal's metabolic effort. Thermoregulation and dissipation mechanisms are activated with higher THI on a daily basis and with high intensity from morning to afternoon, stabilizing versus evening with hourly basis inside the barn. Even if significant relationships were found among temperature (and THI) increases and animal variables, respiration rate and heart reate were observed as those with broader variation indicating that they are more adapt to be used as markers pof heat stress at field level.

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2.7. References

Aboul-Naga, A.I. 1987. The role of aldosterone in improving productivity of heat-stressed farm animals with different techniques. Ph.D. thesis. Faculty of Agriculture, Zagazig University, Zagazig, Egypt.

Alexiev, J., Gudev, D., Popova-Ralcheva, S., Moneva, P. 2004. Thermoregulation in sheep. IV. Effect of heat stress on heart rate dynamics in shorn and in shorn ewes from three breeds. J. Anim. Sci. 41, 16–21.

Armstrong, D.V. 1994. Heat stress interaction with shade and cooling. J. Dairy Sci. 77, 2044–2050. https://doi.org/10.3168/jds.S0022-0302(94)77149-6.

Baida, B.E.L., Baumert, M., Kushwaha, A., Swinbourne, A.M., Leu, S.T., van Wettere, W.H.E.J. 2022. Validation if indwelling vaginal sensor to monitor body temperature in ewes. Anim. Biotelemetry. 9,1–15. https://doi.org/10.1186/s40317-022-00278-y.

Brasil, L.H.A., Wechesler, F.S., Júnior, F., Gonçalves, H.C., Bonassi, I.A. 2000. Efeitos do Estresse Térmico Sobre a Produção, Com-posição Química do Leite e Respostas Termorreguladoras de Cabras da Raça Alpina. Rev. Bras. Zootec. 29, 1632–1641. https://doi.org/10.1590/S1516-35982000000600006.

Brown, D.L., Morrison, S.R., Bradford, G.E. 1988. Effects of ambient temperature on milk production of Rubian and Alpine goats. J. Dairy Sci. 71, 2486–2490. https://doi.org/10.3168/jds.S0022-0302(88)79835-5.

El-Sherif, M.M.A., Assad, F. 2001. Changes in some blood constituents of Barki ewes during pregnancy and lactation under semi-arid conditions. Small Rum. Res. 40, 269–277. https://doi.org/10.1016/S0921-4488(01)00174-2.

Fahmy, S. 1994. Effect of crossing Romanov with Rahmani sheep on some physiological and productive performance. M.Sc. thesis. Faculty of Agriculture, Al-Azhar University, Cairo, Egypt.

Finocchiaro, R., Van Kaam, J.B.C.H.M., Portolano, B., Misztal, I. 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.

Gaughan, J., Cawdell-Smith, A.J. 2015. Impact of climate change on livestock production and reproduction. In Climate change impact on livestock: adaptation and mitigation; Sejian, V., Gaughan, J., Baumgard, L., Prasad, C.; Springer, New Delhi, India, pp. 51–60.

George, W.D., Godfrey, R.W., Ketring, R.C., Vinson, M.C., Willard, S.T. 2014. Relationship among eye and muzzle temperatures measured using digital infrared thermal imaging and vaginal and rectal temperatures in hair sheep and cattle. J. Anim. Sci. 92, 49–55. https://doi.org/10.2527/jas.2014-8087.

Gupta, M., Kumar, S., Dangi, S.S., Jangir, B.L. 2013. Physiological, Biochemical and Molecular Responses to Thermal Stress in Goats. Int. J. Livest. Res. 3, 27–38.

Hamadeh, S.K., Rawda, N., Jaber, L.S., Habre, A., Abi Said, M., Barbour, E.K. 2006. Physiological responses to water restriction in dry and lactating Awassi ewes. Livest. Sci. 101, 101–109. https://doi.org/10.1016/j.livprodsci.2005.09.016.

Hamzaoui, S., Salama, A.A.K., Caja, G., Albanell, E., Flores, C., Such, X. 2012. Milk production losses in early lactating dairy goats under heat stress. J. Dairy Sci. 95, 672–673.

Jackson, P.G.G., Cockcroft, P.D. 2002. University of Cambridge, Cambridge, UK. Clinical Examination of Farm Animals, Wiley-Blackwell, Osney Mead, Oxford, UK.

Johnson, H.D. 1987. In: Johnson, H.D. (Ed.), Bioclimatology and the Adaptation of Livestock. Elsevier Science, Publishers BV5, Amsterdam, Oxford, New York, Tokyo.

Joshi, B.C., McDowell, R.E., Sadhu, D.P. 1968. Effect of drugs on sweating rates in Hariana cattle. J. Dairy Sci. 51, 905–909. https://doi.org/10.3168/jds.S0022-0302(68)87101-2.

Kliber, H.H. 1964. (University of Missouri, Columbia, Missouri, United States). (Agricultural Experiment Station, Columbia, Missouri, United States). Research Bulletin, LXVII.

Marai, I.F., El Darawany, A.A., Fadiel, A., Abdel-Hafez, M.A.M. 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.

Mascarenhas, N.M.H., Furtado, D.A., Fonsêca, V.F.C., Souza, B.B., Oliveira, A.G., Morais, F.T.L., Silva, R.S., Silva, M.R., Batista, L.F., Dornelas, K.C., Bezerra, C.V.C., Silva, J.A.P.C. 2023. Thermal stress index for native sheep. J. Therm. Biol. 115, 103607. https://doi.org/10.1016/j.jtherbio.2023.103607.

Mcmanus, C., Paludo, G.R., louvandini, H., Gurgel, R., Sasaki, L.C.B., Paiva, S.R. 2009. Heat tolerance in brazilian sheep: physio-logical and blood parameters. Trop. Anim. Health. 4, 95–101. https://doi.org/10.1007/s11250-008-9162-1.

Mili, B., Chutia, T. 2021. Adaptive Mechanisms of Goat to Heat Stress. In Goat Science-Environment, Health and Economy; Kukovics, S.; Intech Open, London, UK, pp. 1–16. https://doi.org10.5772/intechopen.96874.

NRC. 2007. Nutrient Requirements of Small Ruminants, Sheep, Goats, Cervids, and New World Camelids. National Academy Press, Washington, DC.

Peana, I., Fois, G., Cannas, A. 2007a. 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.

Peana, I., Dimauro, C., Carta, M., Gaspa, M., Fois, G., Cannas, A. 2007b. Effects of heat stress on milk yield in Sardinian dairy sheep farms. Ital. J. Anim. Sci. 6, 581 (Abstract). https://doi.org/10.4081/IJAS.2007.1S.535IJAS.2007.1S.581.

Peana, I., Francesconi, A.H.D., Dimauro, C., Cannas, A., Sitzia, M. 2017. Effect of winter and spring meteorological conditions on milk production of grazing dairy sheep in the Mediterranean environment. Small Rumin. Res. 153, 194–208. https://doi.org/10.1016/j.smallrumres.2017.06.016.

Rashamol, V.P., Sejian, V., Bagath, M., Krishnan, G., Archana, P.R., Bhatta, R. 2018. Physiological adaptability of livestock to heat Stress: an updated review. J. Anim. Behav. Biometeorol, 6, 62–71. http://dx.doi.org/10.31893/2318-1265jabb.v6n3p62-71.

Sano, H., Ambo, K., Tsuda, T. 1985. Blood glucose kinetics in whole body and mammary gland of lactating goats exposed to heat stress. J. Dairy Sci. 68, 2557–2564. https://doi.org/10.3168/jds.S0022-0302(85)81137-1.

Sejian, V., Bhatta, R., Gaughan, J., Malik, P.K., Naqvi, S.M.K, Lal, R. 2017. Adapting Sheep Production to Climate. In Sheep Production Adapting to Climate Change, 1st ed.; Sejian, V., Bhatta, R., Gaughan, J., Malik, P.K., Naqvi, S.M.K, Lal, R., Eds.; Springer Nature: Singapore, Volume 1, pp. 1–29. https://doi.org/10.1007/978-981-10-4714-5_1.

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

Sevi, A., Annicchiarico, G., Albenzio, M., Taibi, L., Muscio, A., Dell'Aquila, S. 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.

Shalaby, T.H. 1985. Performance and adaptation of local sheep to varied environmental and managerial conditions. Ph.D. thesis. Faculty of Agriculture, Cairo University, Cairo, Egypt.

Shinde, A.K., Sejian, V. 2013. Sheep husbandry under changing climate scenario in India: an overview. Indian J Anim Sci. 83, 998–1008

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.

Thwaites, C.J. 1985. Physiological responses and productivity in sheep. In Stress Physiology in Livestock (ed. Yousef, M.K.), CRC Press, Inc., Boca Raton, Florida, USA, pp. 25–39.

Turner, H.G. 1982. Genetic variation of rectal temperature in cows and its relationship to fertility. Anim. Sci. 35, 401–412. https://doi.org/10.1017/S0003356100001094.

2.8. Tables

Table 1. Effects of THI on study variables.

					P-value				
THI class, °F	<70	70-75	76-80	>80	THI	Milk yield	Hour	Day	Animal
Resp. rate, act/min	85.23^{a}	94.13^{a}	123.97^{b}	140.57^{c}	<.0001	<.05	<0.01	0.10	0.03
Heart rate, beat/min	58.64^{a}	80.07^{b}	86.70^{b}	87.49^{b}	<.0001	0.17	0.02	0.2	0.06
Rectal temp., °C	39.36^{a}	39.54^{b}	39.72^{c}	39.81^{d}	<.0001	<.0001	0.01	0.06	0.04
Vaginal temp., °C	39.43^{a}	39.57^{a}	39.72^{b}	39.8^{b}	<.0001	<.0001	0.03	0.06	0.04
Ear temp., °C	37.13^{a}	37.33^{a}	37.7^{b}	37.93^{b}	<0.001	0.41	<0.01	0.06	0.04
Udder temp., °C	38.3^{a}	39.09^{b}	39.52^{c}	39.59^{c}	<.0001	<.05	0.42	0.08	0.02

Table 2. Effects of air temperature (AT) on study variables.

					P-value				
AT class, °C	<23	23-25	26-28	>28	AT	Milk yield	Hour	Day	Animal
Resp. rate, act/min	79.45^{a}	91.18^{b}	118.73^{c}	144.69^{d}	<.0001	<0.01	<0.01	0.08	0.03
Heart rate, beat/min	64.45^{a}	79.45^{b}	84.35^{b}	91.38^{b}	<.0001	0.15	0.04	0.39	0.06
Rectal temp., °C	39.36^{a}	39.46^{a}	39.7^{b}	39.86^{c}	<.0001	<.0001	0.03	0.05	0.04
Vaginal temp., °C	39.49^{a}	39.52^{a}	39.7^{b}	39.82^{c}	<.0001	<.0001	0.05	0.06	0.04
Ear temp., °C	37.1^{a}	37.27^{a}	37.7^{b}	37.9^{b}	<.0001	0.39	<.01	0.09	0.04
Udder temp., °C	38.38^{a}	39.13^{b}	39.55^{c}	39.58^{c}	<.0001	<.05	0.2	0.05	0.02

2.9. Figures

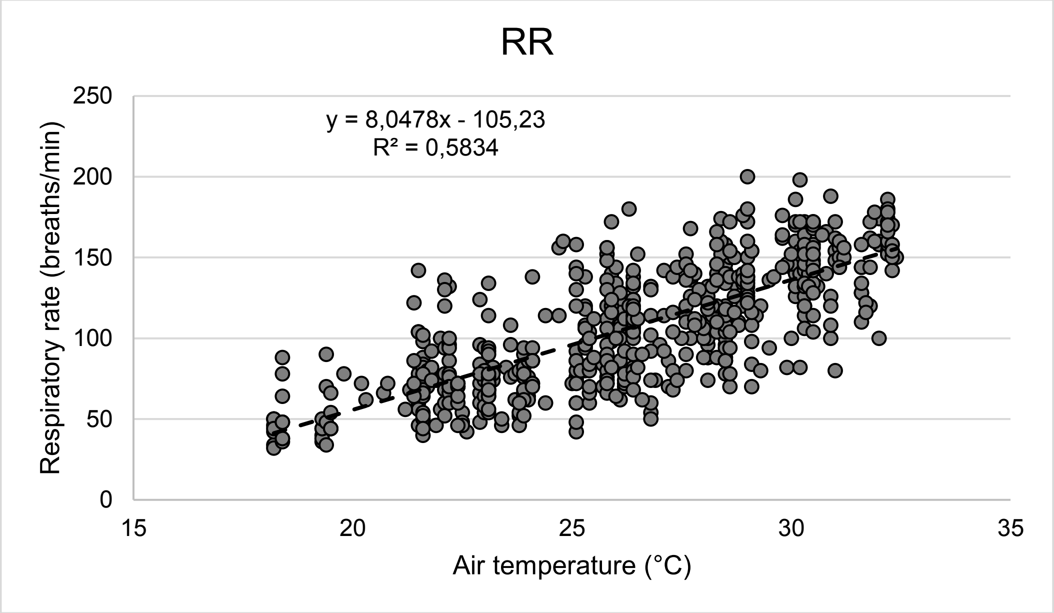


Figure 1. Linear regression between RR (breaths/min) and AT (°C).

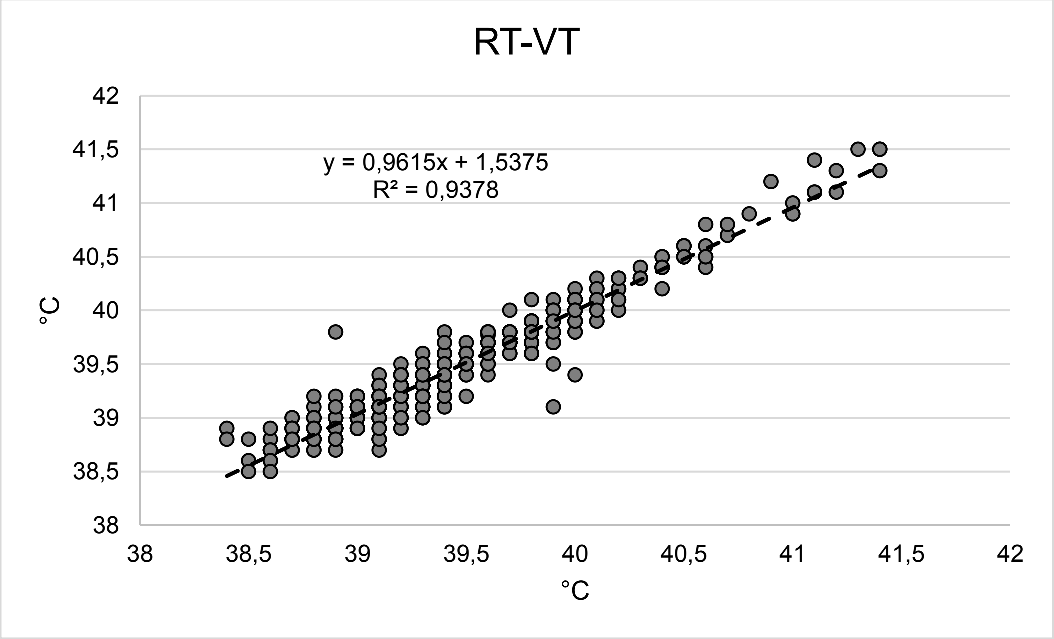


Figure 2. Linear relationship between RT and VT (°C).

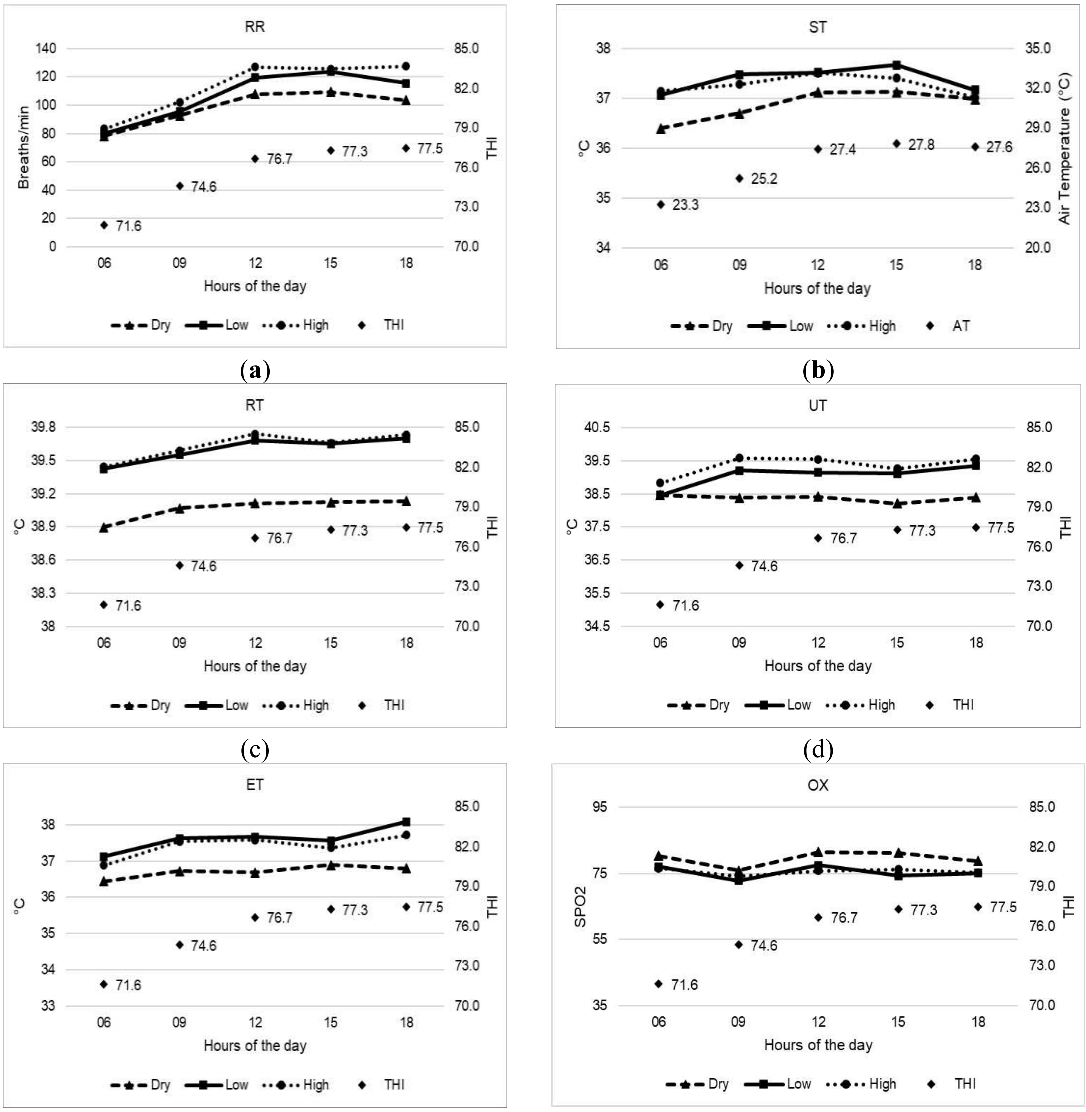

(**a**) (**b**)
(c) (d)
(e) (f)

Figure 3. Daily variation of physiological parameters in relationship to THI: (a) respiratory rate (breaths/min); (b) skin temperature (°C); (c) rectal temperature (°C); (d) udder temperature (°C); (e) ear temperature (°C); (f) blood oxygen saturation (SPO2).

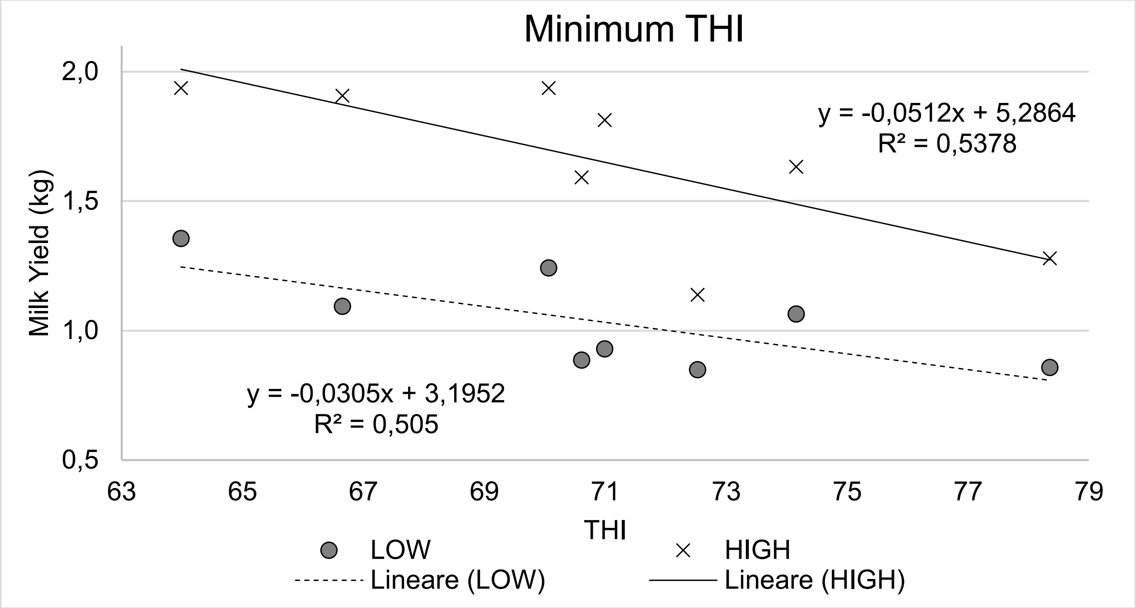


Figure 4. MY variations in relationship to the different THI values.

CHAPTER 3

Comparison of sheep response to environmental temperature using WINTER vs. SUMMER records

3.1. Abstract

Heat and summer conditions cause a large series of changes that involve physiology, metabolism, feeding behavior, reproductive and productive response (Sejian et al., 2017). It is difficul to separate direct short term animal response to heat in term of milk production or changes in single variables (like respiration rate or heat rate) without consider the entire cascade of effects that influences the whole animal response. Objective of this study was to compare markers of heat stress in sheep from summer to winter retrieving data from two experiments carried out in the same experimental barn, with similar experimental conditions and design, in two different seasons (winter 2022 and summer 2021) in order to quantify the ranges of respiration rate and rectal temperatures in dry Sarda sheep breed. RR and RT were not significantly affected by AT in winter (Table 2), showing low values and small standard deviations, equal to 27.8 ± 7.2 and 38.86 ± 0.55, respectively. In summer, RR and RT were equal to 71.1 ± 34.8 and 38.95 ± 0.30, respectively. The RR is considered the best marker of temperature variation, from a minimum of 18 breaths/min at 9°C to a maximum of 174 breaths/min at 32°C. The capacity of the animal to reduce body temperature in summer was equal to -0.05 °C with additional 23 breaths/min. Daily variation of RR and RT indicates tha animals have important accumulation of heat from morning to evening even with medium THI values.

3.2. Introduction

The production of sheep's milk in the Mediterranean Basin, Middle East, Central and Eastern Europe regions, plays a very important role in worldwide milk production, with an estimated economic value of approximately $5,600 million US (FAOSTAT, 2014). Mediterranean area is characterized in general by dry and hot summers and by wet and mild winters, with specific characteristics depending on the region (Ramón et al., 2016). Sardinia (24,090 km2) is the region with the highest concentration of dairy sheep (about 3,020 million). The most important relevant breed is the Sarda sheep, characterized by a good rusticity, longevity, fertility, and an excellent aptitude for grazing. In general ewes are characterized by a remarkable adaptation to climate change (Sejian et al., 2017). During summer occurs heat stress conditions with high variations of daily temperatures (Sevi and Caroprese, 2012; Peana et al., 2017), that increase, on dairy sheep, the internal heat production preventing to maintain thermal homeostasis (Sejian et al., 2017). Respiratory rate with rectal temperature are good indexes of heat stress condition of animals and they are considered as the most important measures even of physiological status (Rashamol et al., 2018). In fact, animals start a series of physiological mechanism to lose the excessive amount of body heat that involves respiration and vapour heat exchange. At 35°C the humidity loss from breath increases for approximately 60% in sheep (Thompson et al., 1985) and the body temperature can increase until to compromise the performances and the health of animals. Heat and summer conditions cause a large series of changes that involve physiology, metabolism, feeding behavior, reproductive and productive response (Sejian et al., 2017). It is difficul to separate direct short term animal response to heat in term of milk production or changes in single variables (like respiration rate or heat rate) without consider the entire cascade of effects that influences the whole animal response. One of the core mechanisms adopted by the animals is the reduction of feed intake in order to decrease the metabolism heat production, it consequentially reduces the transport of nutrients for the synthesis of milk. The reduction of metabolic heat production is totally guaranteed by the decrease of feed intake (Kadzere et al., 2002). Other mechanisms include the enhancement of mechanisms for heat dissipation rates for the thermoregulation homeostasis. All those mechanisms act togheter and is difficult to understand what are the basal conditions totally unaffected by heat stress just considering only summer animal performances and characteristics.

Respiration rates for adult sheep are 20-30 breaths/min (Jackson and Cockcroft, 2002). Marai et al. (2007) observed that respiratory rates during summer increase in sheep through the day (from 08:00h to 15:00h). Normal rectal temperatures for sheep ranges between 38.3-39.9°C. and with the increase of the air temperature from 18°C to 35°C as consequence the rectal temperature increase (Marai et al., 2007). With a rectal temperature of 42°C the animal risks his life (Thwaites, 1985). With the exposure of sheep to heat stress, the whole metabolism of protein, energy, mineral balance, enzymatic reactions, hormonal secretions and blood metabolites are compromised. It would be beneficial to consider differences among summer and winter to analyze the animal characteristics separately or in comparison.

Even grazing time in sheep (minute of grazing/hour of the day) was significantly higher from 7:00 to 11:00 than 11:00 to 15:00 and from 15:00 to 19:00 during the day and in winter (20.1-11.4°C), than in summer (35.4-18.9°C). On the contrary water intake was significantly higher at 11:00 to 15:00 and 15:00 to 19:00 than 7:00 to 11:00 (in particularly in summer) (Marai et al., 1997; Marai et al., 2000).

Objective of this study was to compare markers of heat stress in sheep from summer to winter retrieving data from two experiments, carried out in the same experimental barn with similar experimental conditions and design, in two different seasons(winter 2022 and summer 2021).

3.3. Material and methods

3.3.1. Animals, diet and intakes

Two experiments were conducted in the experimental farm of the Department of Agriculture of University of Sassari located in the north-west of Sardinia (Sassari, Italy; 40°48’41.4” N 8°17’50.7” E). All procedures involving animals were fully in compliance with the European Community (86/609) and Italian regulations (DPR 27/1/1992, Animal Protection Regulations of 124 Italy) on animal welfare and experimentation.

Thirty-two Sarda dairy ewes in dry – non pregnant physiological stage, ranging from 45 to 59.3 kg of full body weight (BW) in summer 2021 (July 19 to August 13; Atzori et al., 2023) and sixteen other sheep (BW between 39.2 and 51.9 kg) in winter 2022 (January 3 to February 11) have been selected from a larger group of ewes in a healthy status to be involved in experimental activities. Animals were housed in metabolic cages with limited physical activity and were monitored for their response to variations of air temperature. In the first experiment (summer 2021) animals were fed with a dry total mixed ration (TMR) based on 70% hay and 30% concentrate mix; in the second experiment (winter 2022) were fed with only dehydrated alfalfa hay (Table 1). Animals were fed 3 times per day in summer and 2 times per day in winter period, collecting the residues of the previous meal before each new administration; the water was given ad libitum. Feed ingredients and chemical composition of diets were presented in Table 1. Experimental measures on metabolic cages, included records and collection of offered and residual meals, used to calculate dry matter intake per each meal for each animal, and total fecal and urine collection and sampling. The total feed residuals and total feces and urines were weighted individually. In particular, feed residues and feces were collected each day before each meal administration, weighted, mixed, proportionally subsampled (20% of their total fresh weight) and immediately stored at -20°C until chemical analysis. Water intake was measured daily per each cage.

3.3.2. Physiological measures

The variables studied were the respiratory rate (RR), measured with visual counts of respiratory acts detected in two videos of 1 minute each, and rectal temperature (RT) measured using a digital thermometer (model: VedoFamily by PIC Solution). Physiological and weather records were gathered six times per day in summer (8:30, 12:30, 16:30, 20:30, 00:30 and 4:30) and four times per day in winter (7:30, 11:30, 15:30 and 19:30).

3.3.3. Temperature Humidity Index (THI)

THI, the index that combined effects of air temperature and relative humidity associated with thermal stress, as reported by Peana et al. (2017) was calculated using Kliber's equation (1964): $\boldsymbol{THI = (1.8\,AT - \left(\left(1 - \frac{RH}{100}\right)(AT - 14.3)\right) + 32}$; where AT is air temperature and RH is relative humidity. Whether variables were measured inside the barn using a whether station (PCE Italia s.r.l., PCE-FWS 20N, Lucca, Italy).

3.3.4. Statistical analyses

Statistical analyses were performed with SAS (v.9.2) testing a mixed model for repeated measurements considering dependent variables as animal measures of respiration rate, rectal temperature; as independent variables classes of air temperature (AT) or THI; season and hours as main effects; animal and day as random effects. The body surface/volume ratio, estimated based on biometrics measures, was also included as covariate.

3.4. Results

3.4.1. Respiratoy rate and rectal temperature

RR and RT were not significantly affected by AT in winter (Table 2), showing low values and small standard deviations, equal to 27.8 ± 7.2 and 38.86 ± 0.55, respectively. In summer, RR and RT were equal to 71.1 ± 34.8 and 38.95 ± 0.30, respectively. Generally, RT and RR were positively associated with AT ($P < 0.0001$; Table 1). In Figure 1 is represented the generic trend of RR from the cold period to the hot. With the increase of air temperature, it was registered a linear raise of breaths frequency per minute, going from a minimum of 18 breaths/min at 8.9°C to a maximum of 174 breaths/min at 31.6°C. A regression equation was developed explaining the 58% of whole RR varaibility:

RR, acts/min = 40.7 + 0.00246 AT (°C) - 12.8 Season + 2.72 Surface/Volume (R^2 = 0.58).

It must be noticed that AT explained the 56% of the diurnal variability of the RR, 1% was explained by season (being winter = 0 and summer = 1 in the equation) and 1% by the body surface/volume ratio ($P < 0.001$). Furthermore, the effect of season can be quantified in +12.8 act/minute in summer vs. winter. Figures 2 and 3 show the different animal adaptations in the two seasons, along the hours of the days. In the Figure 2 is represented the daily variation of RR, from 7:00 to 19:00 for the winter period (left part of the graph) and from 0:00 to 20:00 for the summer period (right part). During winter's temperatures it was registered an insensible variation of number of breaths/min. With a variation of 3.5 °C from the coldest hour of the day to the hottest, the number of acts increase of about 0.1 breaths/min. Summer daily variation, showed a marked swing of acts number starting from 44.5 acts/min (with 24.2 °C at 4:00 AM) to 90.6 acts/min (with 30.1 °C at 16:00) on average. In the Figure 3 it is represented the RT variation (with the same hour points during the day). In winter period, RT ranges from 38.7 °C (with 11.1°C at 7:00 AM) to 38.8 °C (with 14.6 °C at 15:00) and showing an increae of +0.1 °C from morning to afternoon, despite the fact that the air temperature is lower, 12.5°C, at 19:00). During the summer period RT decrease from 39.0°C (with 24.2 °C at 4:00 AM) to 38.8 °C (with 29.1 °C at 12:00) and then increases to 39.0 °C from 16:00 to 0:00.

3.4.2. Dry matter intake, water intake and excretions

Intake and excretions were studied considering the variation of THI and Surface/Volume ratio (Table 2). Four classes of THI (2 for each season) were created. Statistical analysis showed, generally, that DMI, water intake, fecal and urine excretions were positively associated with THI even in animals were in the same physiological status (respectively $P<0.01$ for DMI; $P<0.001$ for the other three variables). Also, the ratio Surface/Volume influenced the variation of feed and water intake ($P < 0.01$) and the variation in urine excretions ($P < 0.0001$) whereas fecal excretion resulted significantly higher in winter ($P = 0.05$) (Table 3). Figure 4 shows the changes in feed and water intake considering the two season and the various ranges of THI. About winter period it was possible to observe an increase of DMI from 800 g/d to 920 g/d for 53 and 55 points of THI, respectively. Moving to high THI values, the intake decreased of 350 g/d per each increase in 1 degree of THI. The consumption of water in the same period ranged between 2.5 to 3.2 l/d (the maximum values at THI=55). During the summer period, DMI was 1.08 kg/d (with THI=75) and increased to 1.15 kg/d with THI = 76-77. After this treshold, the ingestion suffers a decrease of 200 g/d at THI>83. On the contrary, water intake increased until 3.18 l/d at THI>83.

In addition, has been reported: i) graph showing the regression between rectal temperature and respiratory rate during winter (a) and summer (b) period (Figure 5). It showed that variation of respiratory rate is much broader than rectal temperature, orvery wide even in the same range of rectal temperature indicatind that RR might be a beter proxy for heat stress in the barn environment for the short term conditions; ii) the regression between respiratory rate vs. rectal temperature and water intake vs. dry matter intake (DMI) during the entire experimental period (Figure 6a and 6b respectively). In particular, water consumption was estimated in 2.55 liters per each kg of DMI in dry ewes (Figure 6b in supplemental material).

3.5. Discussions

The results of the presented work confirmed numerous previous observations on sheep about physiological responses to heat stress. About RR this work confirmed that hot environments (Mascarenhas et al., 2023; Shilja et al., 2016) and AT strongly affected his trend, especially during the various moments of the day in summer. Otherwise, in winter animal close to 27.8 ± 7.2 in line with the values ranging from 20 to 30 breaths/min, indicated by Jackson and Cockcroft (2002), or without large variations during the hours of the day; in summer the daily trend is completely different. As observed by other studies (Hamzaoui et al., 2013; Marai et al., 2007; Phulia et al., 2010) the transition by low temperature in the first hours of the day (4:00) to the coldest hours

(12:00-16:00) the rate increases linearly (+7.8 breaths/min for each degree acquired by the environment). RT daily variation showed a different trend. The gradually increase from the lowest temperature of winter period (11.1°C at 7:00) to highest (14.6°C at 15:00), suggest the accumulation of body heat is influenced by the external environment from a season to another; in addition, going from the hottest moment to the final part of the day it is observed another increase of body heat. This phenomenon is probably explained by the real efficacy of the most important mechanism of thermoregulation in the short-term period. On the opposite, during summer period all the dissipation mechanisms are activated and the body temperature decreases by 0.05 °C for each +23 breaths/min. Generally, considering the gradual exposure to increasing temperatures, it is possible to confirm the findings of previous studies (Marai et al., 2007; Shalaby, 1985; Hamzaoui et al., 2013), showing that body temperature tends to increase proportionally and linearly. The study suggests, as observed by other works (Monty et al., 1991; Nardone et al., 1991), that the exposition to heat stress conditions influence negatively the DMI (Indu and Pareek, 2015) and positively water consumption (Marai et al., 1997; Marai et al., 2000; Shilja et al., 2016). As observed by Kawashti et al. (1969), high THI caused an increase in urine volume excretion and, as observed by Silanikove (2000), a reduction of fecal water losses. It might be related with the internal retention of liquids, minerals and metabolites.

3.6. Conclusion

This trial allowed to define respiration rate and rectal temperature ranges for Sarda dairy sheep. Those values ranges from 27 in conditions of no heat stress (<12 °C) and 85 in summer under heat stress condition (28-32 °C). Hourly variation is very important in summer indicating body heat accumulation from morning to evening. In fact, maximum values of individual respiration rate reached 170 breath/minutes at 32 °C. The capacity of the animal to reduce body temperature in summer was equal to -0.05 °C with additional 23 breaths/min.

3.7. Reference

Atzori A.S., Porcu M.A., Fulghesu F., Ledda A., Correddu F. 2023. A dietary blend of essential oils and polyphenols to reduce methane emissions in small ruminants. Anim. Prod. Sci. 63(15), 1483–1493 https://doi.org/10.1071/AN23070

FAOSTAT. 2014. Statistical Database of the Food and Agriculture Organization of the United Nations. Accessed Jun. 13, 2014.

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

Indu, S. and Pareek, A. 2015. A Review: Growth and Physiological Adaptability of Sheep to Heat Stress under Semi –Arid Environment. IJETST. 2(9), 3188-3198. http://dx.doi.org/10.18535/ijetst/v2i9.09Jackson, P., Cockcroft, P. 2002. Clinical Examination of Farm Animals. Wiley-Blackwell, Osney Mead, Oxford, UK.

Kawashti, I.S., Haggag, G.M., Ganem, Y.S. 1969. Comparative study of some physiological responses of two breeds of sheep to Egyptian coastal desert climate. Bull. Fac. Sci. Cairo University. 4, 43.

Marai, I.F.M., Shalaby, T.H., Bahgat, L.B., Abdel-Hafez, M.A. 1997. Fattening of lambs on concentrates mixture diet alone without roughages or with addition of natural clay under subtropical conditions of Egypt. Growth performance and behaviour. In: Proceedings of International Conference on Animal Production & Health, Dokki, Cairo, Egypt.

Marai, I.F.M., Bahgat, L.B., Shalaby, T.H., Abdel-Hafez, M.A. 2000. Fattening performance, some behavioural traits and physiological reactions of male lambs fed concentrates mixture alone with or without natural clay, under hot summer of Egypt. Ann. Of Arid Zone. 39, 449–460.

Marai, I.F., El Darawany, A.A., Fadiel, A., Abdel-Hafez, M.A.M. 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

Mascarenhas, N.M.H., Furtado, D.A., Fonsêca, V.F.C., Souza, B.B., Oliveira, A.G., Morais, F.T.L., Silva, R.S., Silva, M.R., Batista, L.F., Dornelas, K.C., Bezerra, C.V.C., Silva, J.A.P.C. 2023. Thermal stress index for native sheep. J. Therm. Biol. 115, 103607. https://doi.org/10.1016/j.jtherbio.2023.103607.

Monty, D.E., Kelly, L.M., Rice, W.R. 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

Nardone, A., Ronchi, B., Valentini, A. 1991. Effects of solar radiation on water and food intake and weight gain in Sarda and Comisana female lambs. In: Animal Husbandry in Warm Climates, vol. 55. EAAP Publication, pp. 149–150.

Peana, I., Francesconi, A.H.D., Dimauro, C., Cannas, A., Sitzia, M. 2017. Effect of winter and spring meteorological conditions on milk production of grazing dairy sheep in the Mediterranean environment. Small Rumin. Res. 153, 194–208. https://doi.org/10.1016/j.smallrumres.2017.06.016

Phulia, S.K., Upadhyay, R.C., Jindal, S.K., Misra, R.P. 2010. Alteration in surface body temperature and physiological responses in Sirohi goats during day time in summer season. Indian J. Anim. Sci. 80, 340–342.

Ramón, M., Díaz, C., Pérez-Guzman, M.D., Carabaño, M.J. 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

Rashamol, V.P., Sejian, V., Bagath, M., Krishnan, G., Archana, P.R., Bhatta, R. 2018. Physiological adaptability of livestock to heat Stress: an updated review. J. Anim. Behav. Biometeorol. 6, 62–71. http://dx.doi.org/10.31893/2318-1265jabb.v6n3p62-71

Sejian, V., Bhatta, R., Gaughan, J., Malik, P.K., Naqvi, S.M.K., Lal, R. 2017. Sheep production adapting to climate change. Singapore Springer.

Sevi, A. and Caroprese, M. 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

Shalaby, T.H. 1985. Performance and adaptation of local sheep to varied environmental and managerial conditions. Ph.D. thesis. Faculty of Agriculture, Cairo University, Cairo, Egypt.

Shilja, S., Sejian, V., Bagath, M., Mech, A., David, C., Kurien, E., Varma, G., Bhatta, R. 2016. Adaptive capability as indicated by behavioral and physiological responses, plasma hsp70 level, and pbmc hsp70 mrna expression in Osmanabadi goats subjected to combined (heat and nutritional) stressors. Int. J. Biometeorol. 60, 1311–1323.

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

Thompson, G.E. 1985. Respiratory system. In Stress Physiology in Livestock (ed. Young, M.K.), CRC Press, Inc., Boca Raton, Horida, USA, pp. 155–162.

Thwaites, C.J. 1985. Physiological responses and productivity in sheep. In Stress Physiology in Livestock (es. Yousef, M.K.), CRC Press, Inc., Boca Raton, Florida, USA, pp. 25–39.

3.8. Tables

Table 1. Chemical composition of diets used in the two seasons.

Summer 2021		Winter 2022	
Feed included in diet	g/kg	Feed included in diet	g/kg
Grass hay	390	Alfalfa dehydrated hay	1000
Corn meal	150	-	-
Alfalfa hay	130	-	-
Soybean meal 48%	105	-	-
Beet pulp	82	-	-
Wheat straw	80	-	-
Molasses	56	-	-
Mineral integration	7	-	-
Chemical parameters		Chemical parameters	
Dry matter, on as fed basis	879.6	Dry matter, on as fed basis	896.5
Crude protein, on DM basis	150.5	Crude protein, on DM basis	186.4
NDF, on DM basis	446.0	NDF, on DM basis	395.7
ADF, on DM basis	321.0	ADF, on DM basis	270
ADL, on DM basis	65.4	ADL, on DM basis	75.4
Starch+sugars, on DM basis	222.9	Starch+sugars, on DM basis	70
Fat, on DM basis	22.2	Fat, on DM basis	21.6
Ash, on DM basis	75.6	Ash, on DM basis	105.4
NFC, on DM basis	305.7	NFC, on DM basis	325

Table 2. Effect of daily temperature on physiological response of dairy sheep to air temperature variation.

	Winter 2022					Summer 2021			P-value			
AT[1] class, °C	<12	12-16	16-20	20-24	>24	<24	24-28	28-32	AT	Season	Hour	Sup/Vol
RR[2], act/min	26.54^{d}	28.86^{d}	31.19^{cd}	35.01^{bdc}	32.85^{bcd}	47.09^{c}	58.96^{b}	85.22^{a}	<0.001	<0.001	<0.001	<0.001
RT[3], °C	38.68^{c}	38.68^{c}	38.60^{abc}	38.54^{c}	38.37^{c}	39.06^{b}	39.21^{ab}	39.23^{a}	0.01	<0.001	<0.001	<0.001

[1] Air temperature; [2] Respiratory rate; [3] Rectal temperature

Table 3. Effect of THI on physiological response of dairy sheep.

	Winter 2022		Summer 2021		P-value	
THI[1] class, °F	<55	55-58	74-78	>80	THI	Sup/Vol
DMI[2], kg/d	0.86^{a}	0.91^{ac}	1.13^{b}	1.05^{ab}	<0.01	<0.01
Water intake, l/d	2.67	3.06	2.84	3.20	<0.001	<0.01
Fecal ex.[3], kg/d	1.98^{a}	1.49^{ab}	1.36^{ab}	0.79^{b}	<0.001	0.05
Urine ex.[3], kg/d	0.86^{a}	0.78^{a}	1.64^{ab}	2.29^{b}	<0.001	<0.0001

[1] Temperature humidity index; [2] Dry matter intake; [3] Excretion

3.9. Figures

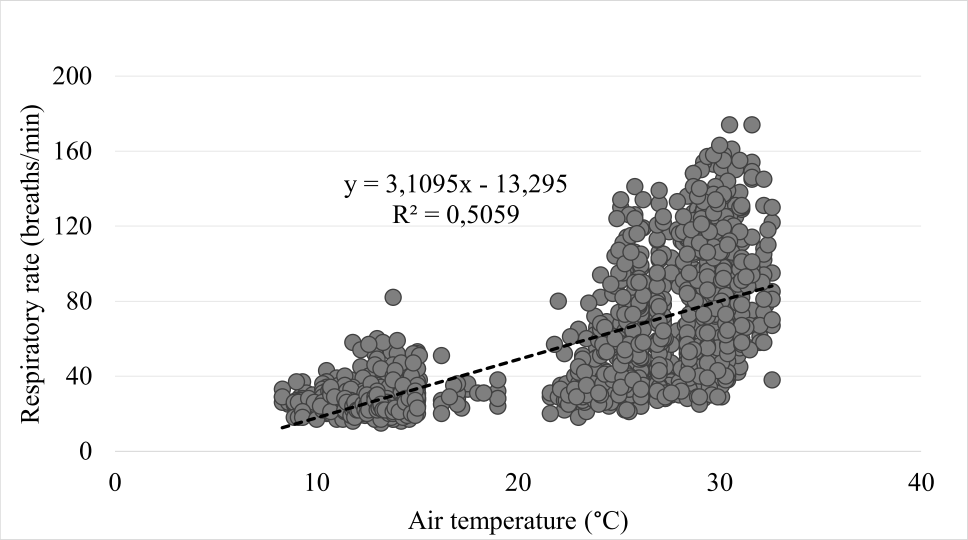


Figure 1. Respiratory rate trend based on the air temperature variation during the two seasons.

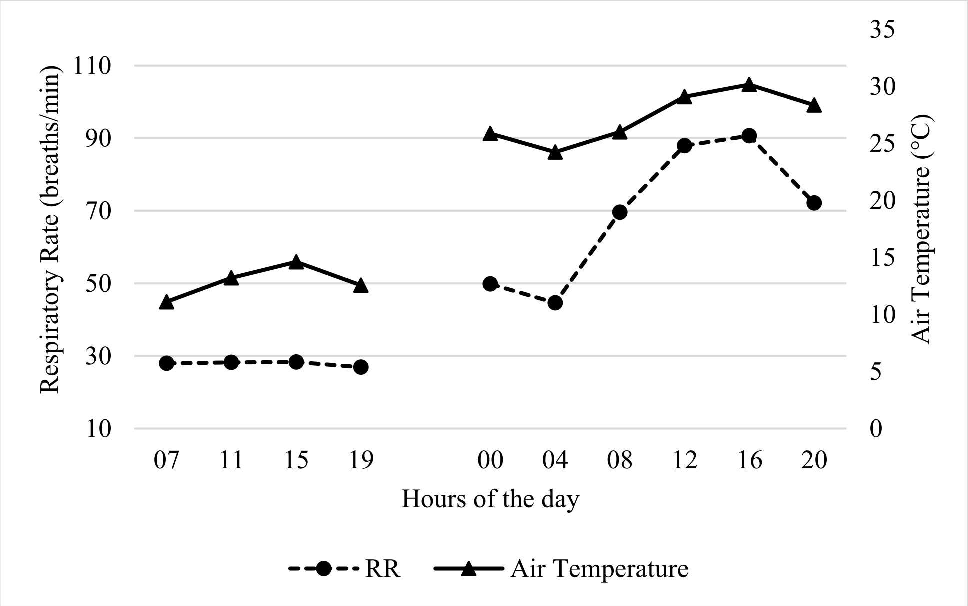


Figure 2. Respiratory rate trend during the hours of the day.

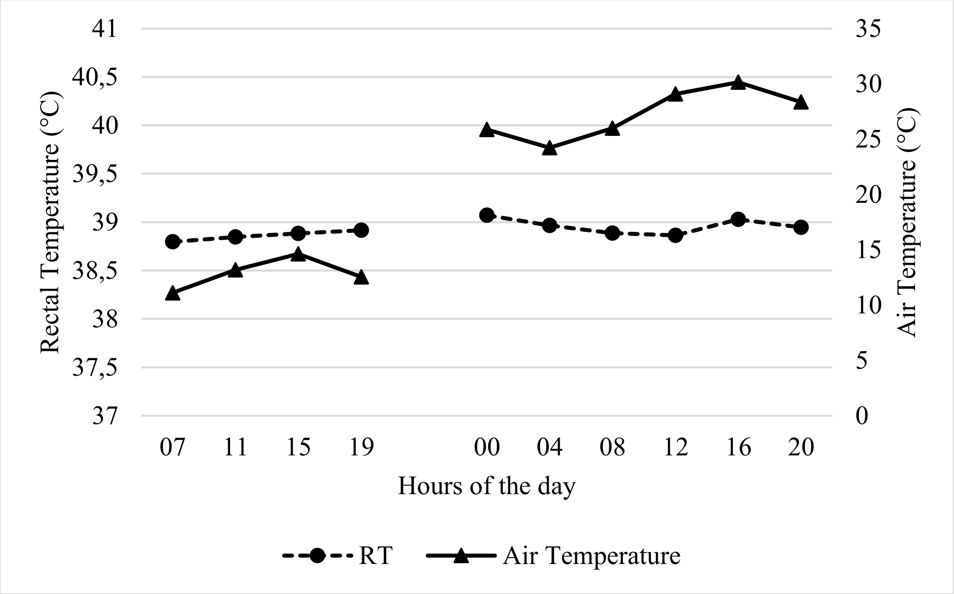


Figure 3. Rectal temperature trend during the hours of the day.

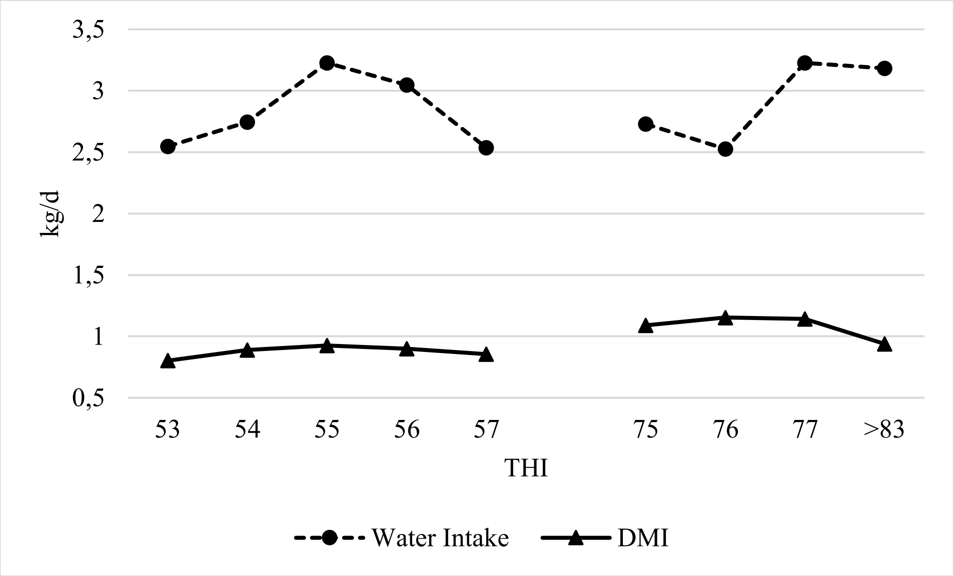
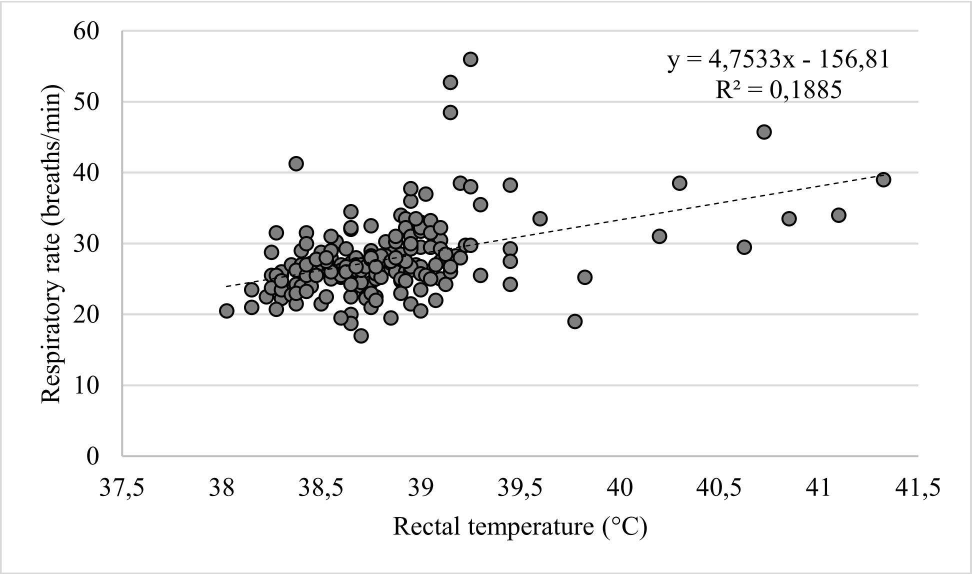
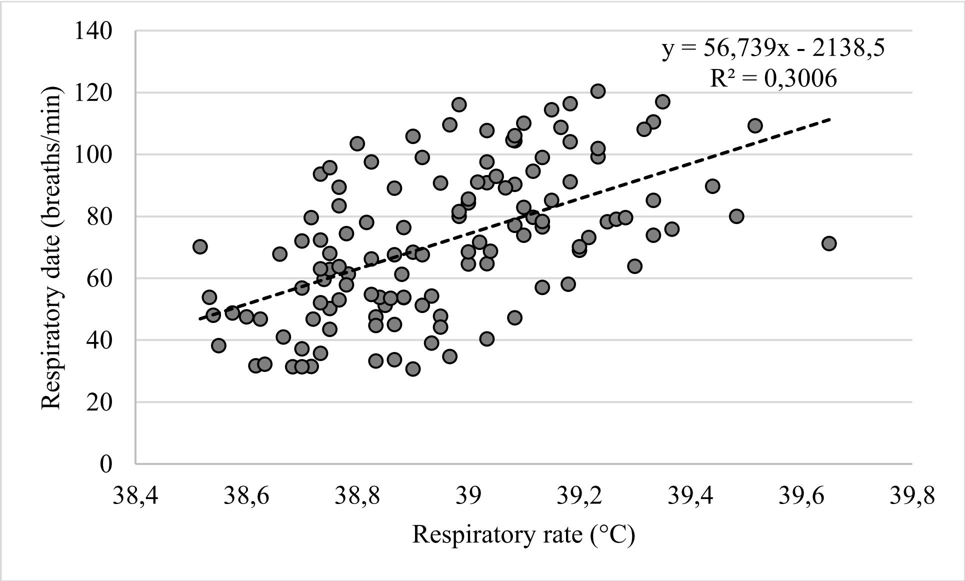


Figure 4. Differences between DMI and Water Intake trends.

Supplemental material

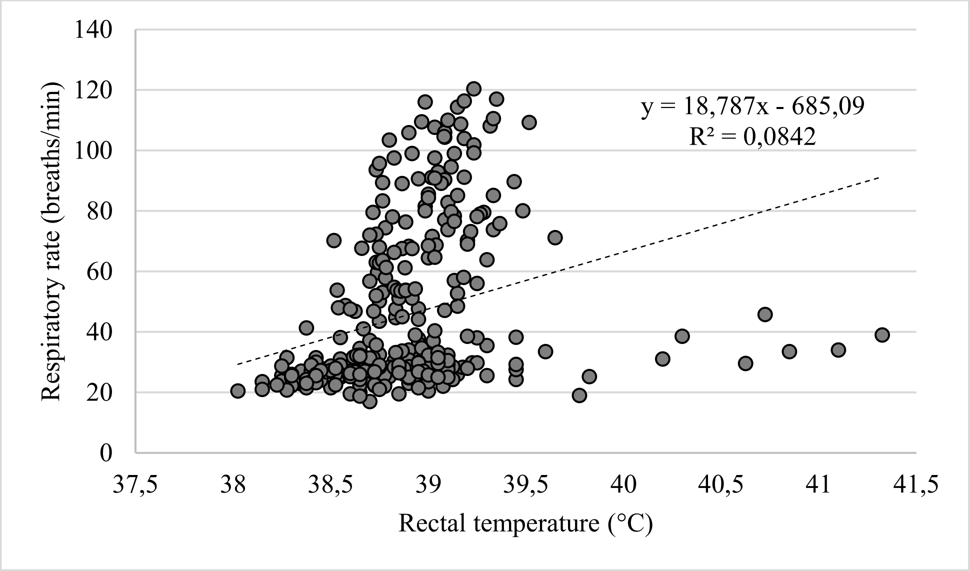


(a)

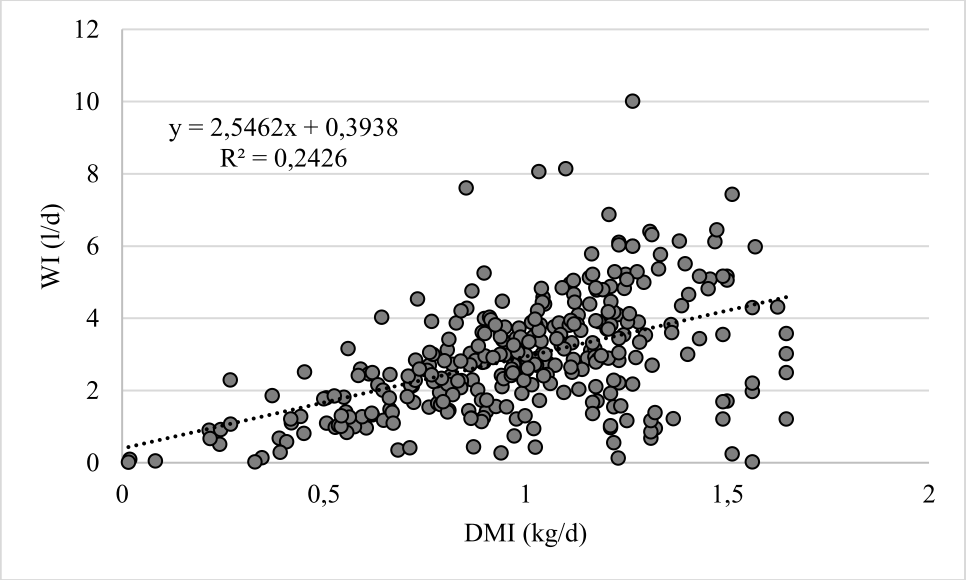


(b)

Figure 5. Regression between rectal temperature and respiratory rate during winter (a) and summer (b) period.



(a)



(b)

Figure 6. Regression between rectal temperature and respiratory rate (a) and dry matter intake (DMI) and water intake (WI) (b) duning the entire experimental period.

CHAPTER 4

Quantification of milk losses and heat stress markers measurable on field on lactating Sarda Sheep housed indoor

4.1. Abstract

As result of reduction of intake and increased energy expenditure for heat dissipation the energy for milk production is reduced and milk losses are common in summer and during heat waves. The aim of this work is to study the effects of heat stress on lactating Sarda dairy sheep intake and milk production and to quantify the effect of heat increase on animal variables measurable at field level. Twenty-one Sarda dairy ewes separated in 3 homogenous groups fed different dfiets for the NDF quality were monitored for 21 days in summer 2022. DMI and MY has been monitored daily and the three physiological parameters were recorded daily 3 times/d at 7.00, 13.00, 19.00 on 4 animals per group. The respiration rate or RR, ocular globe or PT and rectal temperature or RT. DMI was not affected by THI. MY showed a strong decrease with THI>76 (-11.2% compared to THI=71-72; p<0.05) without differences among groups. RT and moreover RR were statistically influenced by THI (p<.0001). RR ranged from 30-50 breaths/min (at THI of 65) to a max of 90-170 breaths/min (at THI of 80) As predictable by the equation: $RR = 0.2555^{\wedge 0.0739*THI}$. On average 41 breaths/min were related to the increase of 1 degree in RT (Figure 1b). It has to be noticed that the measure of RT can be accurately estimated from PT, being a non-invasive method. Additional focus needs to be oriented on the wind speed effect on animal performance and heat stress markers in hot environment.

4.2. Introduction

Heat stress influences physiological aspects, feeding behavior and productive responses. With high environmental temperatures, feed intake of sheep is reduced as well as digestibility and utilization efficiency (Popoola et al., 2014). An increase of intake produces additional endogenous heat that would tend to increase exponentially with critical environmental conditions (Kadzere et al., 2002). For these reasons, the animal decreases the dry matter intake, reducing automatically the level of productivity and adopting a set of physiological responses in order to dissipate internal heat: i) sweating and panting and increases of respiration rate, that are the two automatic primary responses of an animal under heat stress (Gaughan and Cawdell-Smith, 2015); ii) vasodilatation of skin capillary bed and consequently increased the blood flow to the skin surface to facilitate heat dissipation (McManus et al., 2009) even at udder level reducing the nutrients available for milk sysntesis; iii) the increase of pulse rate (Jackson et Cockcroft, 2002); and others. Two of the most important markers of heat stress are the respiratory rate (RR; Piccione et al., 2008; Pennisi et al., 2004) and the rectal temperature (RT; Rashamol et al., 2018; Piccione et al., 2008; Marai et al., 2007) that showed a strong variability with the worsening of environmental conditions. As result of reduction of intake and increased energy expenditure forheat dissipation the energy for milk production is reduced and milk losses are common in summer and during heat waves (Peana et al., 2017). In sheep, a significant and marked reduction of milk production has been observed by a study of Peana et al. (2007a). When maximum and mean temperatures were higher than 21-24°C and 15-21°C respectively, milk lossese were up to 15% of milk production (0.30 kg/d per head). A decrease of 20% (0.38 kg/d per head) was observed when THI passed from 60-65 to 72-75. This marked effects of THI on milk yield, is considering challenging in sheep (Nardone et al., 1992) even if dairy sheep and smaller size animals should be less sensitive than cattle to heat stress. Peana et al. (2007ab) showed that milk yield was negatively affected when THI was above 65; Finocchiaro et al. (2005) observed a decrease in milk yield when THI was above 73; Sevi et al. (2001) observed a decrease in milk yield when THI was above 81.

The aim of this work is to study the effects of heat stress on lactating Sarda dairy sheep intake and milk production and to quantify the effect of heat increase on animal variables measurable at field level.

4.3. Materials and Methods

Twenty-one Sarda dairy ewes (5th month of lactation) housed in the experimental farm of the Department of Agriculture of University of Sassari located in the north-west of Sardinia (Sassari, Italy; 40°48’41.4” N 8°17’50.7” E) were divided into three groups balanced for dry matter intake (DMI; 2.22±0.05 kg/d), milk yield (MY; 2.00±0.03 kg/d), body weight (BW; 53.9±2.04 kg) and BCS (2.98±0.08). The barn was equipped with cooling system (ventilation of 1 m/s). One group received dehydrated chopped oat hay ad libitum (OH; 7.3% crude protein, 63.9% NDF on DM basis); the second and third group received dehydrated chopped alfalfa hay of low quality (ALQ; 19.7% crude protein, 43.5% NDF, DM basis) and high quality (AHQ; 23.5% crude protein, 39.0% NDF, DM basis) ad libitum. Wheter station (PCE Italia s.r.l., PCE-FWS 20N, Lucca, Italy) and recorded varaibles. The experiment lasted a total of 30 days in a colletive box, 8 days of adaptation period and 21 days of experimental phase (from the 21st May 2022 to the 21st June 2022). DMI and MY has been monitored daily and the three physiological parameters were recorded daily 3 times/d at 7.00, 13.00, 19.00 on 4 animals per group. The RR was measured with manual counts within videos of 1 minute, whereas ocular globe (PT) and RT using digital thermometers. Statistical analyses were performed with the software SAS (9.0) testing a mixed model for repeated measurements, considering intake, milk and respiration rate as dependent variables; classes of temperatures and THI, and group as independent variables and considered as fixed effects. Day and animal were included as random effect. Only the effects of heat stress using as environmental parameter the THI was analyzed in respect to animal variables excluding the production effects related with dietary tratments.

4.4. Results and Discussion

Statistical analysis showed that DMI and DMI as kg/kg of BW were not affected by THI during the adaptation and experimental periods ($P>0.05$). The DMI per kg of BW decreases numerically with the increase of THI. This is in contrast to studies that found decreases in DMI in sheep subjected to heat stress (Popoola et al., 2014). The wind speed of 1 m/s could have improved the unattractiveness of the diets, hypothesis which gives greater robustness to the studies which have demonstrated an improvement in the intake capacity of farmed animals through the use of ventilation or other systems. Darcan and Güney (2008), with a ventilation of 1h/d found an increase in DMI (+18%), water intake (+7%) and MY (+21%). Indeed, with regard to the latter parameter, in this study oppositely to DMI variable, MY kg/d and kg/kg of BW (but not MY kg/g of DMI) showed a strong decrease with THI>76 (-11.2% compared to THI=71-72; $p<0.05$) without differences among groups (Table 1). This is in agreement with what observed by Peana et al. (2007b), in which ranging from THI of 60-65 to 72-75, lead to a decrease in MY equal to 20%; also with Finocchiaro et al. (2005; THI>73). RR and RT were statistically influenced by THI ($p<.0001$) in accordance with Marai et al. (2007) and the previous work indicated in the previous chapter of this thesis.

In particular RR ranged from 30-50 breaths/min (at THI of 65) to a max of 90-170 breaths/min (at THI of 80) (Figure 1a) as show by the equation:

$$RR = 0.2555^{\wedge 0.0739*THI}$$

where RR is respiration rate in breaths/min.

RT was linearly related with RR with a broad range of variation, indicating that on average 41 breaths/min were related to the increase of 1 degree in RT (Figure 1b) Finally, the elaboration of RT and PT trends, suggested that the measure of RT can be accurately estimated from PT, being a non-invasive method with the equation:

$$RT\ (°C) = 0.698 * PT\ (°C) + 11.909\ (R^2 = 0.68)\ (\text{Figure 2}).$$

Where RT is rectal temperature and PT is ocular globe temperature.

The data presented in this study lead to a lower accuracy and a greater range of error variation compared to the equation published by Marques et al. (2021), equal to:

$$RT\ (°C) = 1.2867 + 1.0199 * PT\ (°C)\ (R^2=0.90)$$

where RT is rectal temperature and PT is ocular globe temperature.

it has to be noticed that data from Marques et al. (2021) had records only in the extreme values of the RT from and were gathered in dairy goats. It should be checked even the tolerance of the digital thermometers used in this experiment.

4.5. Conclusions

The study underlines the variability of respiration rate and rectal temperature in Sarda sheep during summer conditions in housed barns and the relevance of heat stress on depressing milk yield even without effects on dry matter intake and in ventilated barns. Additional focus needs to be oriented on the wind speed effect on animal performance in hot environment.

4.6. References

Darcan, N., Güney, O. 2008. Alleviation of climatic stress of dairy goats in Mediterranean climate. Small Rumin. Res. 74, 212–215. https://doi.org/10.1016/j.smallrumres.2007.02.007

Finocchiaro, R., van Kaam, J.B.C.H.M., Portolano, B., Misztal, I. 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

Gaughan, J., Cawdell-Smith, A.J. 2015. Impact of climate change on livestock production and reproduction. In Climate change impact on livestock: adaptation and mitigation (ed. Sejian, V., Gaughan, J., Baumgard, L., Prasad, C.). Springer, New Delhi, India, pp. 51–60.

Jackson, P., Cockcroft, P. 2002. Clinical Examination of Farm Animals. Wiley-Blackwell, Osney Mead, Oxford, UK.

Kadzere, C. T., Murphy, M.R., Silanikove, N., Maltz, E. 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

Kliber, H.H. 1964. Environmental ohysiology and shelter engineering with special reference to domestic animals: LXVII. Thermal effects of various temperature-humidity combinations on Holstein cattle as measured by eight physiological responses. Agric. Exp. Station Res. Bull. 862. (University of Missouri, Columbia, MO).

Marai, I.F., El Darawany, A.A., Fadiel, A., Abdel-Hafez, M.A.M. 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

Marques, J.I., Leite, P.G., Neto, J.P.L., Furtado, D.A., Lopes, F.F.M., Borges, V.P., Medeiros, G.R., Sousa, W.S. 2021. Estimation of Rectal Temperature of Goats Based on Surface Temperature. Eng. Agrícola. 41(6), 591–598. https://doi.org/10.1590/1809-4430-Eng.Agric.v41n6p591-598/2021

McManus, C., Prescott, E., Paludo, G.R., Bianchini, E., Louvandini, H., Mariante, A.S. 2009. Heat tolerance in naturalized Brazilian cattle breeds. Livest. Sci. 120, 256–264. https://doi.org/10.1016/j.livsci.2008.07.014

Nardone, A., Lacetera, N., Ronchi, B, Bernabucci, U. 1992. Effetti dello stress termico sulla produzione di latte e sui consumi alimentari di vacche Frisone. Prod. Anim. 5, 1–15

Peana, I., Fois, G., Cannas, A. 2007a. Effects of heat stress and diet on milk production and feed and energy intake of Sarda ewes. Ital. J. Anim. Sci. 6 (1), 577–579. https://doi.org/10.4081/ijas.2007.1s.577

Peana, I., Dimauro C., Carta M., Gaspa M., Fois G., Cannas, A. 2007b. Effects of heat stress on milk yield in Sardinian dairy sheep farms. Proceedings of this Symposium. Doi.org/10.4081/IJAS.2007.1S.535IJAS.2007.1S.581.

Peana, I., Francesconi, A.H.D., Dimauro, C., Cannas, A., Sitzia, M. 2017. Effect of winter and spring meteorological conditions on milk production of grazing dairy sheep in the Mediterranean environment. Small Rumin. Res. 153, 194–208. https://doi.org/10.1016/j.smallrumres.2017.06.016.

Pennisi, P., Costa, A., Biondi, L., Avondo, M., Piccione, G. 2004. Influence of the fleece on thermal homeostasis and on body condition in Comisana ewe lambs. Anim. Res. 53, 13–19. https://doi.org/10.1051/animres:2003047

Piccione, G., Lutri, L., Casella, S., Ferrantelli, V., Pennisi, P. 2008. Effect of shearing and environmental conditions on physiological mechanisms in ewes. J. Environ. Biol. 29, 877–880. http: //www.geocities.com/j_environ_biol/

Popoola, M.A., Bolarinwa, M.O., Yahaya, M.O., Adebisi, G.L., Saka, A.A. 2014. Thermal comfort effects on physiological adaptations and growth performance of West African dwarf goats raised in Nigeria. Eur. Sci. J. 3, 275–382.

Rashamol, V.P., Sejian, V., Bagath, M., Krishnan, G., Archana, P.R., Bhatta, R. 2018. Physiological adaptability of livestock to heat Stress: an updated review. J. Anim. Behav. Biometeorol. 6, 62–71. http://dx.doi.org/10.31893/2318-1265jabb.v6n3p62-71

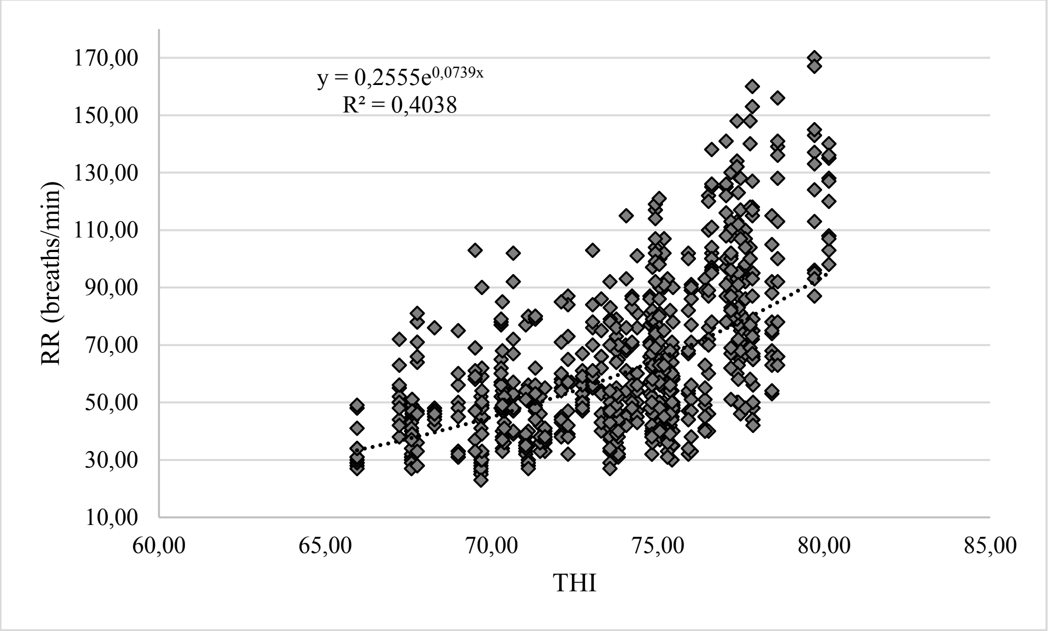
Sevi, A., Annicchiarico, G., Albenzio, M., Taibi, L., Muscio, A., Dell'Aquila, S. 2001. Effects of solar radiation and feeding time on behavior, immune response and production of lactating ewes under high ambient temperature. J. Dairy Sci. 84, 629–640. https://doi.org/10.3168/jds.S0022-0302(01)74518-3

4.7. Tables

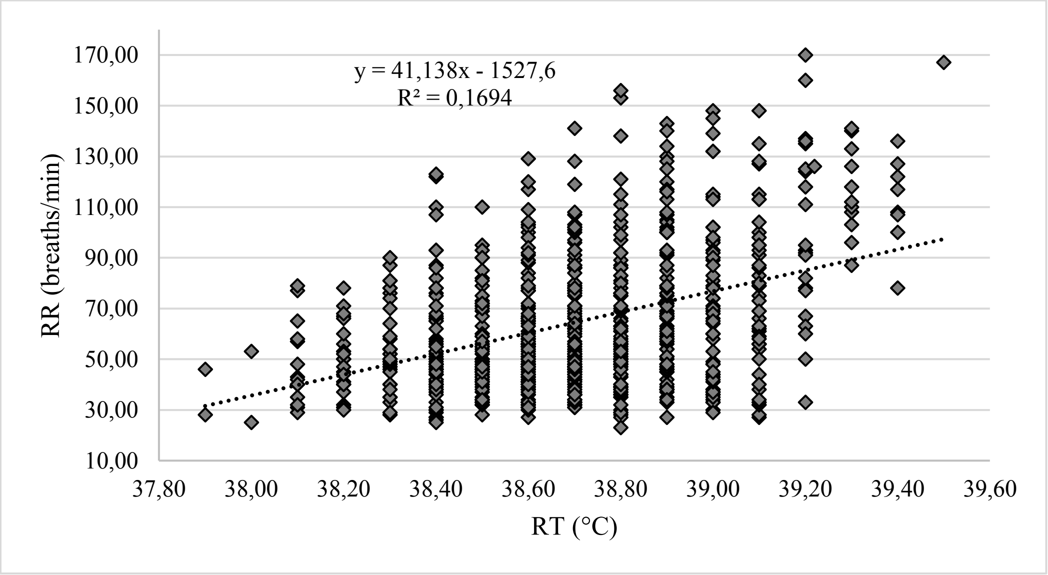
Table 1. Effect of THI variations to physiological response on dairy sheep.

	THI class (F°)					Effects and P-value			
						Fixed		Random	
	<71	71-72	73-74	75-76	>76	THI	Group	Data	ID
DMI, kg/d	2.08	2.07	2.1	2.0	2.1	0.19	<.0001	0.02	0.002
DMI kg/kg of BW	3.88	3.87	3.89	3.62	3.78	0.11	<.0001	0.002	0.03
MY, kg/d	1.79^{a}	1.81^{a}	1.78^{a}	1.73^{a}	1.61^{b}	0.041	0.1394	0.002	0.001
MY kg/kg of BW	33.83^{a}	34.0^{a}	32.89^{a}	31.94^{ab}	29.95^{b}	0.039	0.2637	0.001	0.001
MY kg/g of DMI	0.96	0.94	0.92	0.96	0.90	0.76	<.0001	0.004	0.005

4.8. Figures



(a)



(b)

Figure 1. Correlations between: (a) Respiratory rate (breaths/min) and THI; (b) Respiratory rate (breaths/min) and Rectal temperature (°C).

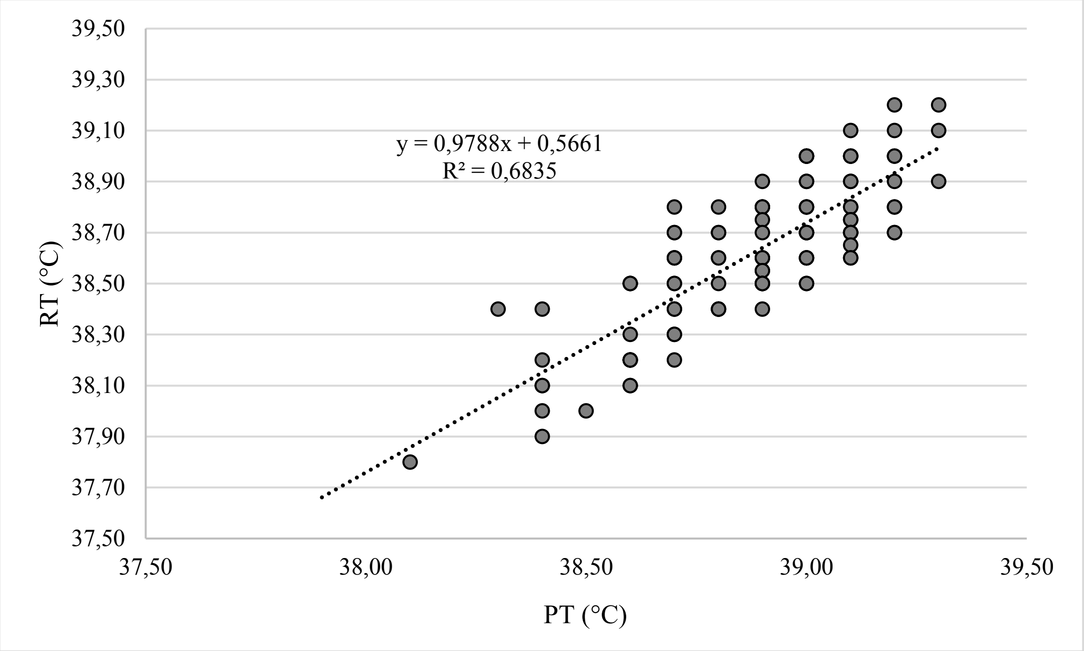


Figure 2. Correlation between Rectal temperature and Ocular globe (PT).

CHAPTER 5

Heat stress measures in sheep housed in metabolic cages and relationship among methane emissions and heat stress markers during two summer trials

5.1. Abstract

Methane emissions direct measurements are carried out in constraining equipment that includes closed environments and constraining space for the animals. The aim of this work was to test the relationships among methane emissions and markers of heat stress, such respiration rate, in a ventilated hood specifically developed for indirect calorimetry calculations and for direct methane emission measurements from sheep. It aims also to undestrand if methane emission measures can be affected by respiration rate during experimental trials. The measurement of methane emission was carried out unindirect calorimetric system designed for small ruminants and based on ventilated hoods. Gas exchange was measured for each animal continuously for 24 hoursonce per week. Intake and milk yield were masured dairy. Respiration rate was measured as described in Chapter 3 and 4. As regards the intake, no significant differences were observed when varying the THI classes. While methane emissions was clearly associated with environmental conditions. Indeed, as THI increased, methane emissions (g/d g/kg BW; g/kg DMI; g/g MY) increased proportionally ($p<0.001$). It was found that methane emissions recorded in equipment for direct measures of methane (ventilated hood) are very sensitive to heat stress and respiration frequency.

5.2. Introduction

The reduction of enteric emissions from ruminants represents a crucial challenge to face global warming (Van Zijderveld et al., 2010). Methane production is a physiological need of ruminant to maintain the reductive potential ruminal environment (Moss, 2002). About 90 % of the methane produced in the gastrointestinal tract of a ruminant occurs at ruminal level and is expelled with eructation, whereas the remaining part is produced in the gross intestine (Murray et al., 1976). Through the fermentation of feed substrate (organic matter, OM), a large amount of hydrogen and electrons are formed inside the rumen and are used by the Archea to reduce CO2 to methane (Hook et al., 2010; Nolan et al., 2010). On the other hand, methane production represents a quite relevant energy loss (from 6 to 10% of gross energy) (Johnson and Johnson, 1995), and its reduction can result in increases in feed efficiency and energy harvesting that might significantly reduce production costs at farm level (Hristov et al., 2013). A large number of in vitro and in vivo trials have been conducted to quantify and measure the methane emission potential of feeds and the effects of bioactive compounds on ruminal fermentation and gas production (Asanuma et al., 1999; Eckard et al., 2010; Bodas et al., 2012; Lee and Beauchemin, 2014). In vitro fermentations allow different diets to be tested simultaneously, alone or in presence of additives and inhibitors, for their effect on methanogenesis (Pirondini et al., 2012). Many additives have been tested to reduce methane production in the rumen, using a wide range of different compounds. Some of them are used as hydrogen acceptors reducing equivalents required for methanogenesis (Lind et al., 2021). Bioactive substances act as rumen modifier such as dietary polyphenols (Vasta et al., 2019) and essential oils (Belanche et al., 2020). Polyphenols, tannins in particular, and essential oils can interfere with rumen microorganisms reducing the methanogenesis. Recent studies showed that the reduction of CH4 production in vivo experiments ranged from 11-30%, with more frequent values around 15-20%. Flavonoids constitute the most characteristic classes of phenolic compounds in plants (Oskoueain et al., 2013). Tannins represent another important class of polyphenols, widely studied. These compounds have shown antimethanogenic effects, mainly due to their effects on the ruminal microbial population, being toxic for some strain of bacteria, protozoa, fungi and archaea (Patra and Saxena, 2011). The effect of polyphenol reduction shows a very large range (in vitro = 4.3% to 70% and in vivo = 6.0% to 68%) (Aboagye and Beauchemin, 2019). Patra et al. (2017) reviewed the effects of a large number of substrates and compounds with antimethanogenic effect. Among them, Essential oils (EO) are obtained from the leaves, flowers, shells, seeds and roots of different aromatic plants by steam distillation or extraction method. Advantages of essential oils are that they are total natural compounds that can be used in very low amount. Several studies have showed that EO can decrease CH4 production without negative effects on feed intake and productivity (Belanche et al., 2020). The same authors in a recent metaanalysis highlighted that few studies have evaluated the effects of EO and their constituents on CH4 emissions in vivo and specific studies are needed to confirm the effect of essential oils blends after the initial adaptation period of the rumen environment (Belanche et al., 2020).

Dairy sheep can be considered a valuable animal model in feeding trials, especially to test productive and metabolic animal response to animal diets. In particular the use of sheep in respect to cows allows: to focus on a large number of animals with lower experimental costs, to take advantage of the higher feeding level of small ruminants, and also to use metabolic cages and chambers to perform accurate measurements and determinations. Direct methane emissions measures performed in equipments specifically developed for indirect calorimetric equipments allow to study the entire energy balance of the animal including energy requirements for maintance and production while estimating direct methane emission from feeding trials (Fernandez et al., 2015). When summer trials are carried out animals can suffer from heat stress and increase their respiration rate. Respiratory rates during summer increase through the day. The number of acts is lower during early morning time (8:00h) than the hot time of the day (15:00h) (Marai et al., 2007). Sheep lose about 20% of total body heat through respiratory moisture in a neutral environment (12°C), and about 60% of total heat at high ambient temperature (35°C) (Thompson, 1985).

The aim of this work was to test the relationships among methane emissions and markers of heat stress, such respiration rate in a ventilated hood specifically developed for indirect calorimetry calculations and for direct methane emission measurements from sheep. It should allow to undestrand if methane emission measures can be affected by respiration rate during experimental trials.

5.3. Materials and methods

5.3.1. Animals and diets

The experiment was conducted in Summer 2022 for 8 weeks (27 June to 13 Agoust) in the experimental farm of the Department of Agriculture of University of Sassari located in the northwest of Sardinia (Sassari, Italy; 40°48’41.4”N 8°17’50.7”E). All procedures involving animals were fully in compliance with the European Community (86/609) and Italian regulations (DPR 27/1/1992, Animal Protection Regulations of 124 Italy) on animal welfare and experimentation. A randomized block design on two parallel groups was designed to test the dietary effect on methane emissions.

Sixteen adult dairy sheep of Sarda breed in milk production – non pregnant physiological stage ranging from 48 to 68 kg of full body weight (BW), have been selected from a larger group of ewes in a healthy status to be involved in experimental activities for 8 weeks. The sheep were randomly allocated to two groups homogenous per BW, both housed indoor under similar conditions, and fed with alfalfa dehydrated hay and corn grain. Feed ingredients and chemical composition of diet were presented in Table 1. One group was randomly maintained as control group (CNT; n = 8; 58.5±9.69 kg of BW; 1.595±0.444 kg of MY), whereases, the other group, assigned as experimental treatment (TRT; n=8; 56.5±8.22 kg of BW; 1.642±0.326 kg of MY) was supplemented with the same bioactive compound premix used by Atzori et al., (2023; Anavrin) consisting of a blend of essential oils, bioflavonoids and chestnut tannins. It consisted of a coated blend of essential oils (EO), mainly from cloves (Syzygium aromaticum), coriander seed (Coriandrum sativum), and geranium (Pelargonium cucullatum), tannins (CT) from chestnuts (Castanea sativa) and bioflavonoids (BF) from olives (Olea europea). The relative concentrations of the active principles were: EO:CT:BF = 1:2.5:0.1. It is produced as commercial product, called Anavrin, by Vetos Europe SAGL, via delle Industrie 18, 6593 - Cadenazzo, Switzerland. During the experimental period, ewes were alternated in 8 cages (4 per group). So the animals stay 7 day in the cage and the next 7 in a collective group. The feed was individually offered to each ewe 2 times a day at 7.00 AM and 7.00 PM and left available for the sequent 11 hours. Feed offered and refusals were weighted for each meal. Diets were offered ad libitum in order to cover the maintenance requirements of each ewe. A big quantity of feed was offered for each round and the availability was monitored during all day (morning and night). Anavrin was included at 1 gram/head/day mixed with 5 grams of corn meal to facilitate the complete ingestion. The other group (Control, CNT) was fed with an amount of 6 grams of corn meal as placebo. The daily amount of Anavrin was administered split in 2 times and provided as oral bolus to each animal, to ensure the individual complete assumption, at 7.00 AM and 7.00 PM during the main meals. Ewes were milked two times per day, at 6.00 AM and 6.00 PM using a portable milker (animals in cages) and using a stationary machine (animals in box). Animals followed this dietary protocol for 28 days, before performing the experimental measurements for methane emissions.

5.3.2. Adaptation to cages, digestibility and methane measurements

After the preliminary phase where the animals were housed in pens, the ewes were transferred to individual metabolic cages, located in the same barn, for the following 7 days. Experimental measures on metabolic cages, included records and collection of offered and residual meals, used to calculate dry matter intake per each meal for each animal, and to perform total fecal and urine collection and sampling. The total feed residuals and total feces and urine were weighted individually during the last 4 experimental days, on which digestibility and methane measurements were carried out. In particular, feed residues and feces and urine were collected each day before each meal administration, weighted, mixed, proportionally subsampled (20% of their total fresh weight) and immediately stored at -20°C until chemical analysis. A pool of the total sampled feces of the 4 days was considered for digestibility determination. Digestibility calculations were carried out for dietary DM, NDF and ADF as percentage of nutrient intake minus nutrient excreted in feces over the nutrient intake. Methane emissions were measured from each ewe after the abovedescribed adaptation period. The measurement of methane emission was carried out using an indirect calorimetric system designed for small ruminants and based on ventilated hoods mounted in the metabolic cages. Hoods were managed in parallel, one for CNT and one for TRT ewes at the same time. Gas exchange was measured for each animal continuously for 24 hours (two periods of 12 hours after the main meals from 7.00 to 19.00 and from 19:00

to 7.00). The ventilated hood system is used in literature to measure methane emissions and to carry out indirect calorimetry experiments (Lind et al., 2021; Criscioni et al., 2016; Fernández et al., 2012, 2015, 2019). The whole system used in this experiment was developed and fully describe by Lai (2020) and by Lind (2021) and consists of two parts: i) two head hoods suspended on the front part of classic metabolic cages for small ruminants, and ii) one air sampling system, which contains instruments for air evacuation and sampling from the hoods and a gas analyzer (GMS810 SICK S.p.A., Vimodrone, MI, Italy) equipped with an internal micro pump and an auto calibration system that allows to keep the system calibrated for a long time. Data from the analyzer were acquired by a software (SOPAS Engineering Tool. SICK S.p.A., Vimodrone, MI, Italy). The ventilated hoods use the same operating principle as the open circuit respiration chamber but are cheaper alternatives (Place et al. 2011). Animals are placed in a metabolic cage which has a separation box where the animals head is positioned (Suzuki et al., 2007). The animal's head is separated by a sleeve of waterproof fabric which guarantee a certain movement to the animal and, at the same time, prevent any air leaks (Bhatta and Enishi, 2007). The head boxes are equipped with fans to move the main air towards the exhaust pipe, the air filters remove humidity, and the gases are led to the analyzer (Suzuki et al., 2007). Airflow is measured and animal's CH4 emission is calculated by analysing for incoming and exhaust air of CH4. Like RC, measurements of CH4 can be obtained continuously.

5.3.3 Measurements

5.3.3.1. Chemical analysis

Feed ingredients, residues and feces collected during the experimental trials were analyzed, in duplicate, for determine chemical composition. Dry matter (DM) was determined by ovendrying samples at 105 °C for 24 h. An Ankom 220 fiber analyzer

(Ankom™ technology, Fairport, NY, USA) was used to determine neutral detergent fiber (NDF) and acid detergent lignin (ADL), following the method of Van Soest et al. (1991). The NDF content was determined using heat stable amylase and expressed exclusive of residual ash; for ADL determination, concentrated sulphuric acid was used to solubilize the cellulose. Crude protein (CP) content was measured according to the Kjeldahl method (proc. 988.05) (AOAC, 2000), extract ether (EE) by the Soxhlet method (proc. 920.39) (AOAC, 2005) and ash by using a muffle at 550°C (proc. 942.05) (AOAC, 2000). Starch was measured by polarimetry (Polax 2L, Atago®, Tokyo, Japan) according to EC (1999). The nonfiber carbohydrates (NFC) were calculated according to Weiss (1999) as follows: NFC (g/kg DM) = 1000 - (NDF + CP + ash + EE).

5.3.3.2. Methane calculations

Calculation of enteric emissions of CH4 was carried out according to Pinares et al. (2012). Accurate measurements of the head hood wet ventilation rate (Wet VR), the net concentration of gas in dry sample, and the percentage of gas recovery in the entire system were considered. According to PinaresPatino et al. (2012), the calculation starts with the results obtained from the gas analyzer, for each point of measurement, expressed as CH4 ppm. The wet ventilation rate (Wet VR) has to be adjusted to dry standard temperature and pressure ventilation rate (Dry STP VR). For a given point of measurement, the emission of CH4 is calculated as follows:

$$\text{CH4 emission (L/min)}=(\text{Dry STP VR}\times([\text{CH4 ppm}]/1000000))/\text{gas recovery rate} \qquad (1)$$

The calculation of dry STP ventilation rate (Dry STP VR) requires data for relative humidity (%), temperature (°C) and pressure (hPa) specific for each head hood system.

Dry STP ventilation rate (L/min)=[(Air pressure×Dry gas VR)/(Chamber T+273.15)]×273.15/1013.25

where pressure is hPa, Dry gas VR is L/min, Chamber T is the chamber temperature in °C.

$$\text{Dry gas VR (L/min)} = \text{Wet VR}\times[(100-\text{VMR})/100] \quad (2)$$

where Wet VR is the ventilation rate recorded from the flow meters in L/min, VMR is the volume mixing ratio of moisture (%).

$$\text{Volume mixing ratio (VMR) (\%)} = 100\times\text{PWP/air pressure} \quad (3)$$

where PWP is the partial water pressure (hPa), and the air pressure in hPa.

The partial water pressure (hPa) is obtained using the Wexler equation:

$$\text{hPa} = (6.1117675 + 0.4439\ T + 0.014305\ T2 + 0.000265\ T3 + 0.00000302\ T4 + 0.0000000204\ T5 + 0.00000000006388\ T6) \times RH/100 \quad (4)$$

where T is head hood temperature (°C) and RH is the chamber relative humidity (%).

Daily emissions can be converted from L/day to g/day using the conversion: 1 g CH4 = 1.3962 L CH4.

5.3.3.3. Nitrogen calculation

The nitrogen content in the urine as well as in the faeces salples was determined by the Kjeldahl method.

5.3.3.4. Respiratpry rate (RR) measurement

The RR was measured with manual counts within videos of 1 minute.

5.3.3.5. Temperature Humidity Index (THI)

THI, the index that combined effects of air temperature and relative humidity associated with thermal stress, as reported by Peana et al. (2017) was calculated using Kliber's equation (1964): $\boldsymbol{THI = (1.8\,AT - \left(\left(1 - \frac{RH}{100}\right)(AT - 14.3)\right) + 32}$; where AT is air temperature and RH is relative humidity. Whether variables were measured inside the barn using a whether station (PCE Italia s.r.l., PCE-FWS 20N, Lucca, Italy).

5.3.3.6. Statistical analysis

Statistical analyses have been carried out using the PROC MIXED of SAS (Version 9.0, SAS Institute Inc., Cary, NC, USA) using the following model:

$$Yijkl = \mu + \alpha i + \beta j + \gamma k + \varepsilon ijkl \qquad (5)$$

Where: Yijkl is the dependent variable (DMI in kg/d, Digestibility of DMI and NDF intake, methane emissions in g/d, g/kg of DMI, gr/kg of BW, g/kg of digested DMI and g/kg of digested NDF intake), μ is the general mean, αi is the main effect of treatment (i=CNT; TRT), βj is the random effect of hood (j=1; 2), γk is the random effect of covariate BW and εijkl is the residual term. Data are expressed as mean ± SEM. The significance of group mean differences was assessed using Tukey honestly significant difference ($P < 0.05$).

5.4. Results and discussion

The groups included a wide range of animal's size from 48 to 68 kg without statistical differences observed between groups for DMI intake, averaging respectively 1297 g/d per ewe in the TRT and 1170 g/d per ewe in CNT groups, on average equal to 2.1% (Table 2). Similar proportions without significant differences were observed for OM intake, NDF intake and ADF intake (Table 2). In the particular case, it seems that the difference was mainly due to forage intake (Table 2); and DMI was in particular higher for TRT vs. CNT specially at the beginning of the trial (Figure 4a).

Animals' diets were offered calculating an amount of DMI, slightly higher than the estimated maintenance requirements as function of the body weight of each ewe. Statistical differences between group performances, attributable to the effect of the dietary treatment were reported in Table 2. The treatment did not affect the diet digestibility which resulted equal to 72.6% and 70.5% of DM respectively for CON and TRT. Similarly, the treatment did not affect the NDF digestibility, which was equal to 43.6% and 49.2% of NDF for CNT and TRT respectively (a lower fiber digestibility as probable effect of higher passage rate and NDF from alfalfa vs. grass hay).

Any significant difference among groups was also observed for OM and ADF digestibility (Table 2). Digestibility of DM and OM and Nitrogen (N) was numerically lower for TRT group vs. CNT group. It indicated that an efficient use of the diet either with or without the premix supplementation was assured. Previous work demonstrated in vitro and in vivo that intake and digestibility were not affected by supplementations with essential oils in vivo trials with sheep (Soltan et al., 2018) and dairy cows (Belanche et al., 2020). No differences were observed among groups in terms of methane emissions expressed as g/d, which resulted equal to 23.2 and 22.9 for the CNT and TRT, respectively (Table 2). The observed results correspond to about 9 kg of methane emitted per ewe per year, in line with the IPCC (2019) and Vermorel (2008) estimates (9.0 and 9.3 kg/head for dry ewes, respectively). The observed values of daily methane emission were similar to the values (24.1 ± 7.6 g/d, mean ± SD) reported by previous works on sheep even if with little difference in DMI intake and breeds among experiments (Bhatt at al., 2019; Jinjer et al., 2016; Knight et al., 2008; Pinare-Patio et al., 2003). The results were totally in line with the previous trial of Atzori et al. (2023) which showed the reduction of methane due to Anavrin use in the short term of 28 days. Emissions expressed per g/kg of BW were very similar among groups (0.423 vs. 0.403). The strong relationship among methane emission and DMI can also been observed looking at the pattern of daily methane emission. Figure 1 reports the means of methane emission during the 24 hours for the two experimental groups. On average, the CNT group showed the highest values in the two moments. These trends result in the not statistical differences among the two groups in term of daily emission, as reported in the Table 2. Independently on the group, the pattern clearly shows the methane peaks closed to the two daily meals (at 07:00 and 19:00). These patterns are in agreement to previous observations in in vivo trials on ovine and bovine (Lai, 2020; Crompton et al., 2011). For that reason, the emissions expressed per kg of DMI or OMI was considered the most adequate way to discuss differences among groups, avoiding individual effects. Emissions expressed per kg of DMI, referred to the unit of substrate fermented at ruminal level, thus directly embedded in the hydrogen formation and methane production. The mean values of methane emission, expressed as g/kg DMI, were closer to those reported by Bhatt et al. (2019) (20.8 vs 24.1). Similar values were observed by Jinker et al. (2016) and Knight et al. (2008) in sheep. Increasing values of emissions observed from week 1 to 7 (Figure 6) were attribute to the variation of the level of intake, which as well known has high influence on ruminal passage rate and fiber retention time, thus fermentation of fibrous substrates that are responsible of methane production (Pinares-Patino, 2011). but without reach significant differences. The inclusion of the premix reduced significantly ($p < 0.05$) the methane emissions per unit of DMI (19.03 and 22.62 g/kg of DMI). These finding shows the ability of the pool of EO, flavonoids and chestnut tannins, to reduce methane emission in ruminants, in agreement to the inhibitory effects of these classes of compounds on methanogenesis, as already observed in previous studies. The extent of the observed reduction was 16%, in agreement to experiments on feeding EO blends to dairy cows in comparable conditions, 13% in Atzori et al., (2023) and 12.9% as reviewed by Belanche et al. (2020), or in respiration chambers (11.0%) as reported by Klop et al. (2017), or in buffaloes (Yatoo et al., 2018), in which has been observed that an EO blend was able to reduce methane production yield by 14% per kg of DMI and digested DMI during six months feeding trial without negative effects on intake and digestibility of dry matter and nutrients in respect to the control diet. Results of the present studies were also in agreement with previous works studying the antimethanogenic effect of dietary polyphenols. Seradj et al. (2014) found that a commercial citrus extract of flavonoids blend reduced methane production, by the inhibition of methanogenic archaea and increasing the concentration of propionate. As far as the effect of chestnut tannins is concerned, inhibitory effect on methane emission of these compounds has been reported in sheep, often without negative effect on growth performance (Liu et al., 2011). However, other works reported no effect of tannin treatment on methane emission from sheep (Wischer et al., 2014).

Several experiments demonstrated that EO blends leads to a decrease in CH4 emissions, with the treatment duration being one of the important factors affecting magnitude and consistency of this reduction. In vitro studies with rumen fluid from donor cows fed EO blends showed only a shortterm effect on CH4 reduction, even with negative effects on DMI and DM digestibility (Klop et al., 2016). In long-term studies the anti-methanogenic effect was consistent across studies leading to a decrease in CH4 production (−8.8%), yield (−12.9%) and intensity per kg of fat and protein corrected milk (−9.9%). It was obtained without affecting feed intake and improving milk production and feed conversion efficiency. In dairy cattle, essential oils tended to decrease the daily CH4 emissions (g/d) and CH4 relative to dry matter intake (g/kg DMI) by 15% and 14%, respectively, after 6 weeks of supplementation (Castro-Montoya et al., 2015). These findings are particularly in line with the presented study for both the long-term effects of methane reductions observed in respect to in vitro studies, and for the extent of emission yield reductions (Oh et al, 1967; Vasta et al., 2019; Cobellis et al., 2016). The main mechanism of action was associated with a changed fermentation pattern that increased propionate (+0.59% units) and decreased acetate molar proportions (-1.0% units) in sheep across 21 studies (Torres et al., 2020). Effects on milk yield are not easy explainable for the following reasons. The number of animals 8+8 is not always enough to test small significant difference in milk yield due to the high daily variation of this variable. Even if groups were balanced for milk yield in the adaptation phase, the animals responded very differently to heat stress and to metabolic cages and hoods adaptation. The animal stress usually causes milk losses and drop, for that reasons statistical analysis of milk production comparisons were performed co-variating the animal performances for the initial milk yield of each animal and the results showed that no significant differences were observed among the two groups (Table 2).

We specifically focused on the THI, it was divided into 3 classes, to test effects related with feed intake, methane emissions and production. As regards the intake, no significant differences were observed when varying the THI classes. While methane emissions was clearly associated with environmental conditions. Indeed, as THI increased, methane emissions (g/d Figure 4d; g/kg BW; g/kg DMI Figure 4c; g/g MY) increased proportionally ($p<0.001$). Milk yields were significantly lower with the transition from a THI of 75-76 to >76 ($p<0.05$; Table 3 and Figure 4b) as expected. As described earlier, in Figure 1, in correspondence with the two feeding times (7:00 and 19:00) there was a significant increase in enteric methane emissions due to particularly intense rumen fermentation activities in the 2-3 hours following feed intake, as expected. From the same graph it is possible to notice how the trend of emissions follows the daily variations of THI (highest in the afternoon hours. In Figure 3a the correlation between CH4 emitted and the THI allowed to develop an equation that explains 40% of the emission variability: CH4 (g/d) = 2.2452 * THI - 151.44 (R^2=0.40). Additional informations were related the N excretions from the analysis of feces and urine collected during the experiment and N intake (Figure 3b). Regarding the levels of feed and water intake, an interesting correlation was observed. In fact, the two levels followed the same trend suggesting a prediction equation equal to: Water intake (g/d) = 3.3873 * DMI (g/d) + 0.2224 (R^2=0.45; Figure 2). Although statistical analysis did not establish a significant difference in DMI levels between the various classes of THI, a numerical reduction of DMI was observed with increasing THI and worsening environmental conditions (Figure 4a). During the experimental trial, the respiratory rate showed that as the THI increases especially during the hottest hours of the day (13:00 h; Figure 5).

5.5. Conclusions

In conclusion, the premix used in this study included in sheep diet (1 gr/d per head) allowed a significant reduction of 16% of methane per kg of dry matter and organic matter intake in comparison to control conditions, without affect digestibility of DM and NDF. The main focus of this experiment in this thesis was the relationship among respiration rate and methane emission in summer conditions. It was found that methane emissions recorded in equipment for direct measures of methane (ventilated hood) are very sensitive to heat stress and respiration frequency. More studies should be carried out to study with indirect calorimetry equipments the nutrient requirement of heat stress.

5.6. References

Aboagye, I.A. and Beauchemin, K.A. 2019. Potential of molecular weight and structure of tannins to reduce methane emissions from ruminants: A review. Animals. 9(11), 856.

AOAC. 2000. Official methods of analysis of AOAC International, 17th edn. Gaithersburg, MD, USA.

AOAC. 2005. Official methods of analysis of AOAC International, 18th edn. Washington, DC, USA.

Asanuma, N., Iwamoto, M., Hino, T. 1999. Effect of the Addition of Fumarate on Methane Production by Ruminal Microorganisms In Vitro. J. Dairy Sci. 82, 780–7.

Atzori A.S., Porcu M.A., Fulghesu F., Ledda A., Correddu F. 2023. A dietary blend of essential oils and polyphenols to reduce methane emissions in small ruminants. Anim. Prod. Sci. 63(15), 1483–1493. https://doi.org/10.1071/AN23070

Belanche, A., Newbold, C.J., Morgavi, D.P., Bach, A., Zweifel, B., Yáñez-Ruiz, D.R. 2020. A Meta-analysis describing the ef-fects of the essential oils blend Agolin Ruminant on performance, rumen fermentation and methane emissions in dairy cows. Animals. 10 (4), 620.

Benchaar, C., Calsamiglia, S., Chaves, A.V., Fraser, G.R., Colombatto, D., McAllister, T.A., Beauchemin, K.A. 2008. A review of plant-derived essential oils in ruminant nutrition and production. Anim. Feed Sci. Technol. 145, 209–228. Doi: 10.1016/j.anifeedsci.2007.04.014

Benchaar, C., Greathead, H. 2011. Essential oils and opportunities to mitigate enteric methane emissions from ruminants. Anim. Feed Sci. Tech. 166–167, 338–355. https://doi.org/10.1016/j.anifeedsci.2011.04.024

Bhatt, R.S, Soni, L., Sahoo, A. 2019. Methane production and microbial protein synthesis in adult sheep fed total mixed ration as mash and as complete feed block. Carbon Manag. 10(3), 241–253.

Bhatta, R., Enishi, O. 2007. Measurement of Methane Production from Ruminants. Asian-Australas. J. Anim. Sci. 20, 1305–1318.

Bodas, R., Prieto, N., García-González, R., Andrés, S., Giráldez, F.J., López, S. 2012. Manipulation of rumen fermentation and methane production with plant secondary metabolites. Anim. Feed. Sci. Technol. 176, 78–93.

Castro-Montoya, J., Peiren, N., Cone, J. W., Zweifel, B., Fievez, V., De Campeneere, S. 2015. In vivo and in vitro effects of a blend of essential oils on rumen methane mitigation. Livest. Sci. 180, 134–142.

Cobellis, G., Trabalza-Marinucci, M., Yu, Z. 2016. Critical evaluation of essential oils as rumen modifiers in ruminant nutrition: A review. Sci. Total Environ. 545–568.

Criscioni, P., Marti, J.V., Pérez-Baena, I., Palomares, J.L., Larsen, T., Fernández, C. 2016. Replacement of alfalfa hay (Medicago sativa) with maralfalfa hay (Pennisetum sp.) in diets of lactating dairy goats. Anim. Feed Sci. Technol. 219, 1–12.

Crompton, L.A., Mills, J.A.N., Reynolds, C.K., France, J. 2011. Fluctuations in methane emission in response to feeding pattern in lactating dairy cows. 7th Int. Workshop Model. Nutr. Dig. Util. Farm Anim. Paris Fr. 10-12 Sept. 2009, 176–80.

Eckard, R.J., Grainger, C., de Klein, C.A.M. 2010. Options for the abatement of methane and nitrous oxide from ruminant pro-duction: A review. Livest. Sci. 130, 47–56.

Fernández, C., López, M.C., Lachica, M. 2012. Description and function of a mobile open circuit respirometry system to measure gas exchange in small ruminants. Anim. Feed Sci. Technol. 172, 242–246.

Fernández, C., López, M.C., Lachica, M. 2015. Low-cost mobile open-circuit hood system for measuring gas exchange in small ruminants: from manual to automatic recording. J. Agric. Sci. 153, 1302–1309.

Fernández, C., Pérez-Baena, I., Marti, J.V., Palomares, J.L., Jorro-Ripoll, J., Segarra, J.V. 2019. Use of orange leaves as a replacement for alfalfa in energy and nitrogen partitioning, methane emissions and milk performance of murciano-granadina goats. Anim. Feed Sci.Technol. 247, 103–11.

Frutos, P., Hervás, G., Giráldez, F.J. and Mantecón, Á.R. 2004. Tannins and ruminant nutrition.

Hassanat, F. and Benchaar, C. 2013. Assessment of the effect of condensed (acacia and quebracho) and hydrolysable (chestnut and valonea) tannins on rumen fermentation and methane production in vitro. J. Sci. Food Agric. 93(2), 332–339.

Hook, S.E., Wright, A-D.G., McBride, B.W. 2010. Methanogens: Methane Producers of the Rumen and Mitigation Strategies. Ar-chaea N. Y. 2010:945785.

Hristov, A.N., Oh, J., Firkins, J.L., Dijkstra, J., Kebreab, E., Waghorn, G., Makkar, H.P.S., Adesogan, A.T., Yang, W., Lee, C., Gerber, P.J., Henderson, B., Tricarico, J.M. 2013. Special topics--Mitigation of methane and nitrous oxide emissions from animal operations: I. A review of enteric methane mitigation options. J. Anim. Sci. 91, 5045–69.

IPCC. 2019. Refinement to the 2006 IPCC Guidelines for National Greenhouse Gas Inventories, Prepared by the National Greenhouse Gas Inventories Programme, Volume 4: Agriculture, Forestry and Other Land Use (AFOLU). Calvo Buendia, E., Tanabe, K., Kranjc, A., Baasansuren, J., Fukuda, M., Ngarize, S. Osako, A., Pyrozhenko, Y., Shermanau, P. and Federici, S. (eds). Published: IPCC, Switzerland.

Jinker, A., Molano, G., Sandoval, E., Taylor, P.S., Antwi, C., Olinga, S., Cosgrove, G.P. 2016. Methane emissions differ between sheep offered a conventional diploid, a high-sugar diploid or a tetraploid perennial ryegrass cultivar at two allowances at three times of the year. Anim. Prod. Sci. 58, 1043–1048.

Johnson, K.A., Johnson, D.E. 1995. Methane emissions from cattle. J. Anim. Sci. 738, 2483–2492. https://doi.org/10.2527/1995.7382483x

Klop, G., Hatew, B., Bannink, A., & Dijkstra, J. 2016. Feeding nitrate and docosahexaenoic acid affects enteric methane production and milk fatty acid composition in lactating dairy cows. J. Dairy Sci. 99(2), 1161–1172.

Klop, G., Van Laar-Van Schuppen, S., Pellikaan, W. F., Hendriks, W. H., Bannink, A., & Dijkstra, J. 2017. Changes in in vitro gas and methane production from rumen fluid from dairy cows during adaptation to feed additives in vivo. Animal, 11(4), 591–599.

Knight, T.W., Molano, G., Clark, H., Cavanagh, A. 2008. Methane emissions from weaned lambs measured at 13, 17, 25 and 35 weeks of age compared with mature ewes consuming a fresh forage diet. Aust. J. Exp. Agric. 48, 240–243.

Lai, F. 2020; PhD Thesis Dissertation, XXXII Cycle University of Sassari, Advisor prof. Atzori A.S.

Lee, C., Beauchemin, K.A. 2014. A review of feeding supplementary nitrate to ruminant animals: nitrate toxicity, methane emissions, and production performance. Can. J. Anim. Sci. 94(4), 557–570.

Lind, V., Schwarm, A., Mele, A., Cappucci, A., Foggi, G., Sizmaz, O., Tsiplakou, E., Atzori, A.S., Van Mullen, J., Peiren, N. 2021. Nutritional approaches to reduce enteric methane emission from ruminants. Chapter 7. In Bartzanas T. and Calvet S. Tech-nology for Envirnomental Friendly Livestock Productions. From LIVAGE COST ACTION 16106. In press.

Liu, H., Vaddella, V., Zhou, D. 2011. Effects of chestnut tannins and coconut oil on growth performance, methane emis-sion, ruminal fermentation, and microbial populations in sheep. J. Dairy Sci. 94(12), 6069–6077.

Marai, I.F., El Darawany, A.A., Fadiel, A., Abdel-Hafez, M.A.M. 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

Moss, A.R. 2002. Environmental control of methane production by ruminants. In: International conference on greenhouse gases and animal agriculture. pp 35–43.

Murray, R.M., Bryant, A.M., Leng, R.A. 1976. Rates of production of methane in the rumen and large intestine of sheep.

Nolan, J.V., Hegarty, R.S., Hegarty, J., Godwin, I.R., Woodgate, R. 2010. Effects of dietary nitrate on fermentation, methane production and digesta kinetics in sheep. Anim. Prod. Sci. 50, 801.

Oh, H. K., Sakai, T., Jones, M. B., Longhurst, W. M. 1967. Effect of various essential oils isolated from Douglas fir needles upon sheep and deer rumen microbial activity. Appl. Microbiol. 15(4), 777–784.

Oskoueian, E., Abdullah, N., Oskoueian, A. 2013. Effects of flavonoids on rumen fermentation activity, methane pro-duction, and microbial population. BioMed research international, 2013.

Patra, A.K. and Saxena, J. 2011. Exploitation of dietary tannins to improve rumen metabolism and ruminant nutrition. J. Sci. Food Agric. 91(1), 24–37.

Patra, A.K., Park, T., Kim, M., Yu, Z. 2017. Rumen methanogens and mitigation of methane emission by anti-methanogenic compounds and substances. J. Anim. Sci. Biotechnol. 8, 13.

Pinares-Patiño, C.S., Ulyatt, M.J., Lassey, K.R., Barry, T.N., Holmes, C.W. 2003. Rumen function and digestion parameters associated with differences between sheepin methane emissions when fed chaffed lucerne hay. J. Agric. Sci. Cambridge. 140, 205–214

Pinares-Patiño, C.S., Ebrahimi, S.H., McEwan, J.C., Dodds, K.G., Clark, H., Luo, D. 2011. Is rumen retention time implicated in sheep differences in methane emission? Proc. N.Z. Soc. Anim. 71, 219–222.

Pinares-Patiño, C.S., Hunt, C., Martin, R., West, J., Lovejoy, P., Waghorn, G.C., Waghorn, G. 2012. New Zealand ruminant methane measurement centre, AgResearch, Palmerston North. Tech. Man. Respir. Chamb. Des. CS Pinares-Patiño GC Waghorn, pp 11–30.

Pirondini, M., Malagutti, L., Colombini, S., Amodeo, P., Crovetto, G.M. 2012. Methane yield from dry and lactating cows diets in the Po Plain (Italy) using an in vitro gas production technique. Ital. J. Anim. Sci. 11:e61.

Place, S.E., Pan, Y., Zhao, Y., Mitloehner, F.M. 2011. Construction and Operation of a Ventilated Hood System for Measuring Greenhouse Gas and Volatile Organic Compound Emissions from Cattle. Animals, 1, 433–46.

Seradj, A.R., Abecia, L., Crespo, J., Villalba, D., Fondevila, M., Balcells, J. 2014. The effect of Bioflavex® and its pure fla-vonoid components on in vitro fermentation parameters and methane production in rumen fluid from steers given high con-centrate diets. Anim. Feed Sci. Technol. 197, 85–91.

Soltan, Y.A., Natel, A.S., Araujo, R.C., Morsy, A.S., Abdalla, A.L. 2018. Progressive adaptation of sheep to a microencapsulated blend of essential oils: Ruminal fermentation, methane emission, nutrient digestibility, and microbial protein synthesis, Anim. Feed Sci. Technol. 237, 8–18.

Suzuki, T., McCrabb, G.J., Nishida, T., Indramanee, S., Kurihara, M. 2007. Construction and operation of ventilated hood-type respiration calorimeters for in vivo measurement of methane production and energy partition in ruminants. In: Eds. Makkar H.P.S., Vercoe P.E. Measuring methane production from ruminants. Springer, pp 125–135.

Tedeschi, L.O., Cannas, A., Fox, D.G. 2010. A nutrition mathematical model to account for dietary supply and requirements of energy and other nutrients for domesticated small ruminants: The development and evaluation of the Small Ruminant Nutri-tion System. Small Rumin. Res. 89, 174–84.

Thompson, G.E. 1985. Respiratory system. In Stress Physiology in Livestock (ed. Young, M.K.), CRC Press, Inc., Boca Raton, Horida, USA, pp. 155–162.

Torres, R.N.S., Moura, D.C., Ghedini, C.P., Ezequiel, J.M.B., Almeida, M.T.C. 2020. Meta-analysis of the effects of essential oils on ruminal fermentation and performance of sheep. Small Rumin. Res. 189, 106148.

Van Soest, P.J., Robertson, J.B., Lewis, B.A. 1991. Methods for Dietary Fiber, Neutral Detergent Fiber, and Nonstarch Polysaccha-rides in Relation to Animal Nutrition. J. Dairy Sci. 74, 3583–97.

Van Zijderveld, S.M., Gerrits, W.J.J., Apajalahti, J.A., Newbold, J.R., Dijkstra, J., Leng, R.A., Perdok, H.B. 2010. Nitrate and sulfate: Ef-fective alternative hydrogen sinks for mitigation of ruminal methane production in sheep. J. Dairy Sci. 93, 5856–66.

Vasta, V., Daghio, M., Cappucci, A., Buccioni, A., Serra, A., Viti, C., Mele, M. 2019. Invited review: Plant polyphenols and rumen microbiota responsible for fatty acid biohydrogenation, fiber digestion, and methane emission: Experimental evidence and methodological approaches. J. Dairy Sci. 102, 3781–804.

Vermorel, M., Jouany, J.P., Eugène, M., Sauvant, D., Noblet, J., Dourmad, J.Y. 2008. Evaluation quantitative des émissions de méthane entérique par les animaux d’élevage en 2007 en France. INRA Prod. Anim. 21, 403–418.

Wischer, G., Greiling, A.M., Boguhn, J., Steingass, H., Schollenberger, M., Hartung, K., Rodehutscord, M. 2014. Effects of long-term supplementation of chestnut and valonea extracts on methane release, digestibility and nitrogen excretion in sheep. Animal, 8(6), 938–948.

Weiss, W.P. 1999. Energy prediction equations for ruminant feeds. In: Proceedings of the 61st Cornell nutrition conference for feed manufacturers (Cornell University, Ithaca). 176–185.

Yatoo, M.A., Chaudhary, L.C., Agarwal, N., Chaturvedi, V.B., Kamra, DN. 2018. Effect of feeding of blend of essential oils on methane production, growth, and nutrient utilization in growing buffaloes. Asian-Australas J Anim Sci. 31(5), 672–676.

5.7. Tables

Table 1. Experimental diet offered to experimental Control (CNT) and Treatment (TRT) groups.

	Alfalfa dehydrated hay	Corn grain
Dry matter%, on as fed basis	89.64	86.3
Crude protein%, on DM basis	18.64	9.8
NDF%, on DM basis	39.57	0.1
ADF%, on DM basis	27	2.8
ADL%, on DM basis	7.54	1
Sugars%, on DM basis	4.97	2
Fat%, on DM basis	2.16	4.3
Ash%, on DM basis	10.54	1.6
NFC%, on DM basis	32.5	79.9

[1] The Treatment group was additionally supplemented with 1g/head/day of Anavrin as reported in the text.

Table 2. Effect of dietary premix on performances, digestibility, and methane emissions in sheep.

ITEM	Treatments			P value			
Performances	CON	TRT	*SEM*	Treatment	Period	Treat x period	Hood
Corn intake, g/d per head	346.2	359.2	10.3	0.54	0.7	0.36	0.66
Alfalfa intake, g/d per head	823.8	937.8	46.5	0.40	0.128	0.884	0.79
Water intake ml/d per head	3340	3519	242	0.71	0.69	0.2	0.4
Dry matter intake, g/d per head	1170	1297	49.4	0.21	0.16	0.74	0.68
Milk Yield, g/d per head*	737.3	742.2	28.9	0.91	0.2	0.54	0.29
Methane							
Methane, g/day per ewe	23.20	22.90	0.68	0.80	0.01	0.73	0.21
Methane, g of CH_4/kg DMI	22.62	19.03	1.04	0.04	0.01	0.52	0.89
Methane CH_4/kg of BW	0.423	0.403	0.02	0.26	0.04	0.60	0.27
Media di CH_4/kg of MILK	35.56	34.60	2.02	0.80	<0.001	0.64	0.93
Digestibility measurements**							
DMI, kg/d	1.676	1.613	52.6	0.57			
Water intake, l/d	4.987	5.508	340	0.20			
NDF, % DM	35.19	33.84	25.0	0.50			
Organic matter (OM), % DM	88.0	88.0	1.24	0.99			
Digestibility of DM, % of DM	72.64	70.54	0.80	0.20			
Digestibility of NDF, % of NDF	49.17	43.57	1.7	0.11			
Digestibility of OM, % of OM	73.58	73.75	0.84	0.92			
Digestibility of N, % of N	84.02	82.80	0.63	0.35			

*Covariate comparison to account for initial differences; **only from samples collected at week 5.

Table 3. Effect of THI on performances and methane emissions in sheep.

ITEM	THI Class				P value		
Performances	75-76	77-78	79-80	SEM	THI	Group	Hood
Dry matter intake, g/d per head	1331.05	1168.05	1123.39	99,08	0.225	0.821	0.373
Water intake ml/d per head	3294.86	3350.79	3830.21	625,6	0.696	0.947	0.453
Methane							
Methane, g/day per ewe	19.14^{a}	24.49^{b}	26.41^{b}	1.47	<.0001	0.8218	0.426
Methane, g of CH_4/kg BW	0.353^{b}	0.443^{b}	0.474^{b}	0.28	0.0002	0.6203	0.278
Methane, g of CH_4/kg DMI	0.015^{a}	0.023^{ab}	0.031^{b}	0.004	0.0011	0.835	0.357
	THI Class				P value		
Performances	75-76	77-78	79-80	SEM	THI	Group	BW
Milk Yield, g/d per head*	837.36^{a}	693.42^{b}	692.17^{b}	44.15	0.003	0.635	0.0105
Methane							
Methane, g of CH_4/g of Milk	0.025^{a}	0.039^{b}	0.0433^{b}	0.004	<.0001	0.853	0.278

* Covariate comparison to account for initial differences

5.8. Figures

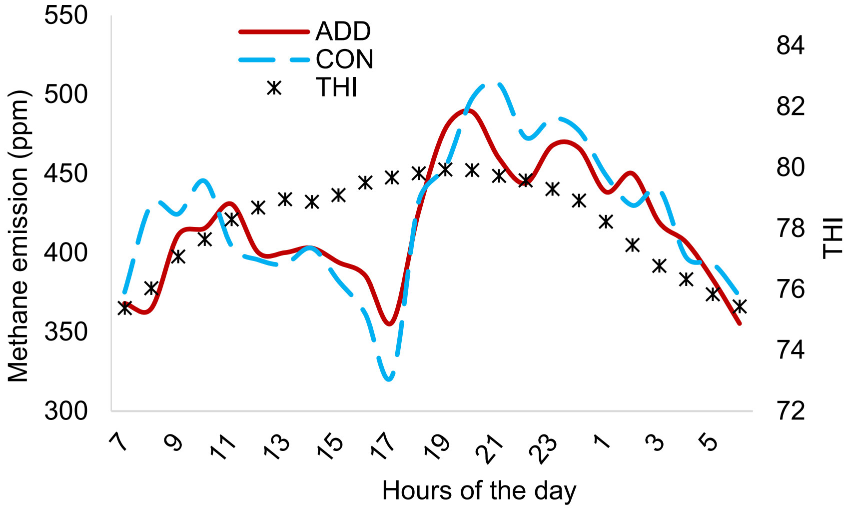


Figure 1. Means of daily emission patterns of animals of the two experimental groups in relationship with THI. The two daily meals were offered at 07.00 and 19.00.

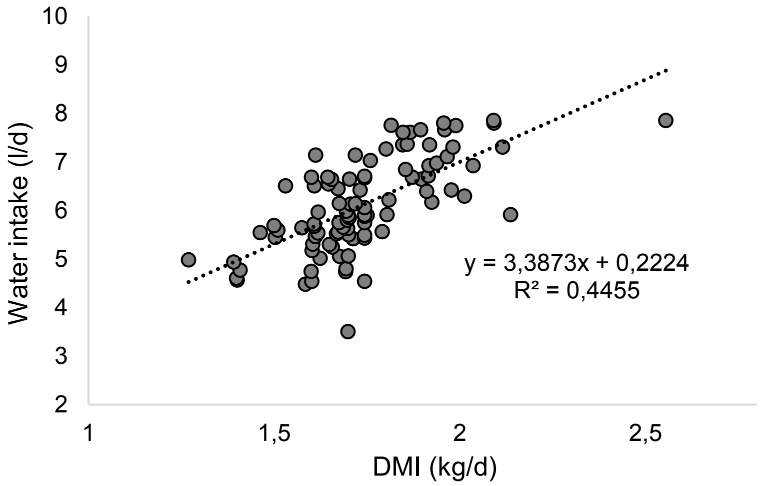
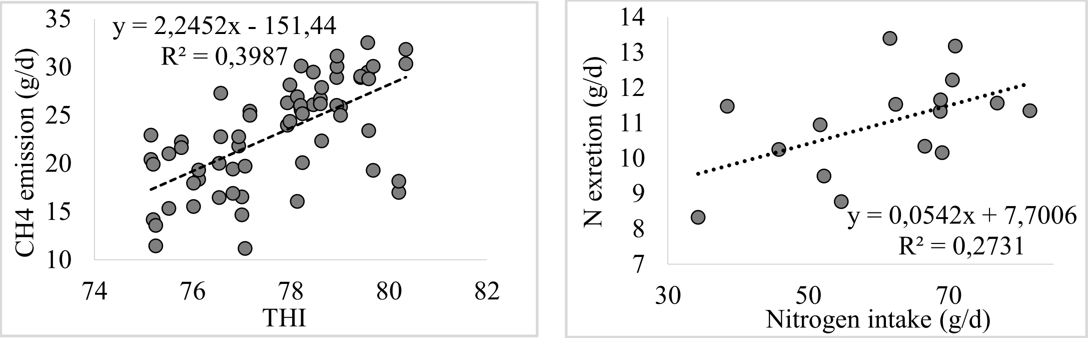
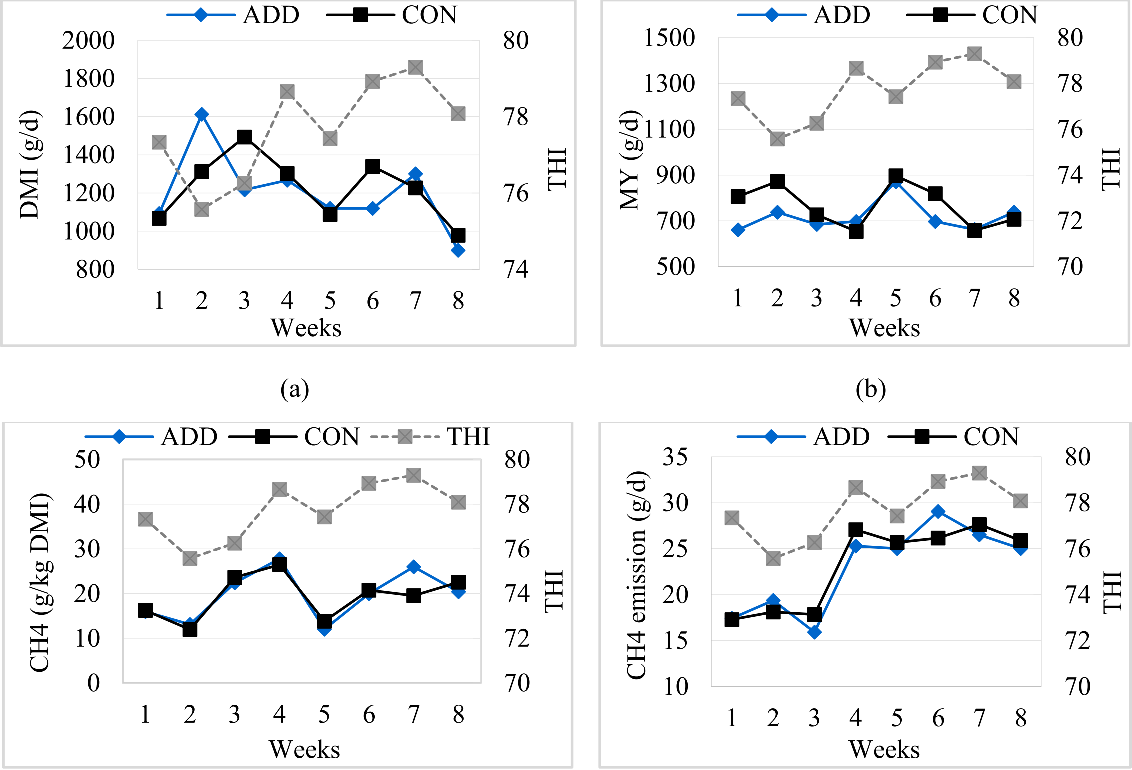


Figure 2. Regression between water intake and DMI.



(a) (b)

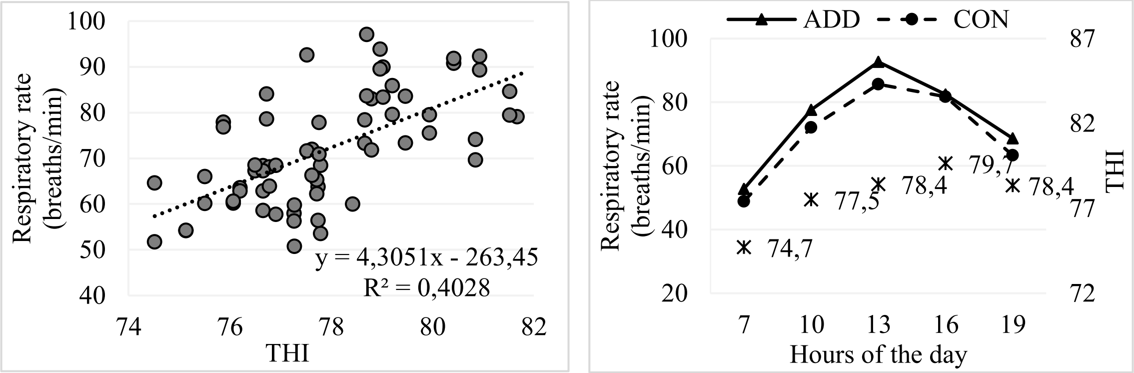
Figure 3. Regression between CH4 emissions (g/d) and THI (a); regression between Nitrogen excretions (fecal and urinary) and Nitrogen intake (b).



(a) (b)

(c) (d)

Figure 4. Variation of animal's performances and CH4 emissions in relationship to THI: (a) DMI (g/d); (b) MY (g/d); (c) CH4 emission (g/kg DMI); (d) CH4 emission (g/d).



(**a**) (**b**)

Figure 5. Regression between RR and THI (a); daily variation of RR in relationship to THI (b).

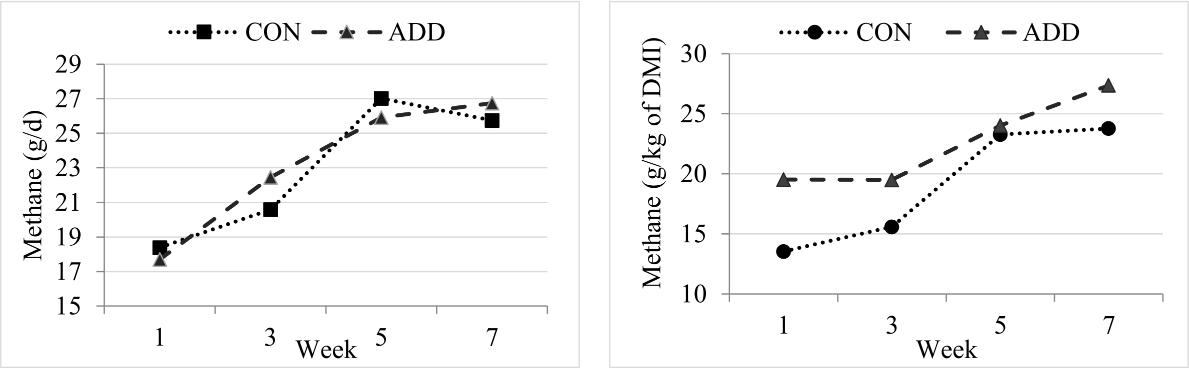


Figure 6. Methane emission expressed in g of CH4/d per head (a); Methane emission expressed in g of CH4/kg of DMI (b).

CHAPTER 6

Preliminary Modelling of heat stress

6.1. Abstract

Animal size is relvant for heat exchange and dissipation, and volume to surface ratio is considered one of the most important scaling factors that affect metabolic weight calculation and maintenance requirements estimations. It is also a target parameter to develop models of heat stress. Empiric models of heat stress have been proposed mainly as algorithms to adjust maintenance requirements of the main feeding systems however dynamic approaches able to capture hat production and dissipation and accumulation delays have not been developed. In this chapter animal biometric measures from the trial described in Chapter 2 were retrieved to develop a geometric model to estimate the surface and volume of sheep. A series of equations were elaborated to estimate volume and surface; chest circunference was the best variable to accurately estimate surface and volume. A system dynamics qualitative model was then developed using the causal loop diagramming tecnicque, to show the structure of a future model that can capture the two main feedback loops of the heat accumulation: heat production due to intake and heat dissipation due to physiological mechanisms. Further studies nee to be carried out to calculate energy expense for heat dissipation mechanisms.

6.2. Introduction

In terms of heat dissipation systems in farm animals, sheep are in an intermediate position between horses and cattle (species in which sweating prevails) and pigs and dogs (in which polypnea is the main means of defense against heat). Under conditions of high ambient temperatures, increased respiration rate is the main method of heat loss. In mammals, respiration is directed at removing CO_2 from body tissues and supplying O2 under thermo-neutral conditions, evaporating moisture from the respiratory tract, and preventing hypothermia at high ambient temperatures. Sheep lose about 20% of total body heat through respiratory moisture in a neutral environment (12°C), and moisture loss increases and accounts for about 60% of total heat loss at high ambient temperature (35°C) (Thompson, 1985). Evaporation becomes the most important pathway for heat dissipation, since sweating in sheep is much less important than respiratory evaporation because thermal regulation is influenced by fleece characteristics, among other factors. Sheep fleece is related to breed, age, sex and environmental conditions, such as temperature, relative humidity and wind. It is also an insulating layer that protects the animal from both heat and cold. The type of fleece is extremely important. The open fleece of dairy breeds provides less protection against solar radiation than the closed fleece of Merino breeds (Pennisi et al., 2004). With long periods of heat exposure, animals develop certain mechanisms to produce less body heat. These mechanisms include decreasing food intake and heat production, as well as stretching the body to lose as much heat as possible (Marai et al., 1997). When the ambient temperature rises to 36°C, sheep ears and limbs dissipate a high percentage of the heat, as these areas contribute about 23% of the body surface area (Johnson, 1987). Heat exchange between animal and environment is an important component of metabolic heat production, in addition to growth, milk production, pregnancy and activity components. The animal's body surface is the interphase for convective, radiant and evaporative heat loss from the skin, supplemented by convective and evaporative heat loss through the respiratory system. Heat is released from the animal by evaporative heat loss and non-evaporative heat loss. Evaporative heat, from the respiratory tract and skin, is relative (but not linearly proportional) to the heat stored in the body. In contrast, non-evaporative heat loss is determined by the animal-environment temperature gradient and the surface area of the animal. Body surface area is a useful parameter for estimating combined convective and radiant heat loss. Body surface area is, in fact, a component of heat transfer models (McGovern and Bruce, 2000; Turnpenny et al., 2000; Gebremehdin and Wu, 2001) and is also used in energy demand models (ARC, NRC, CNCPS) to calculate energy requirements during cold and heat stress conditions. Several studies have led to the formulation of predictive equations relating surface area to body mass: $0.105 \times W^{0.67}$ (Meeh, 1879), $0.09 \times W^{0.67}$ (Mitchell, 1928), $0.14 \times W^{0.57}$ (Brody, 1945), $0.235 \times W^{0.46}$ (Johnson et al, 1961). Some of the equations assume similarity of body proportions with increasing body mass; others are empirical. Equations that assume similarity of body proportions also imply that body shape, as well as body mass density, remain similar as body mass increases. Body surface area estimates are similar at body weight under 100 kg; however, they diverge progressively with increasing weight. The surface area calculated with the $0.105 \times W^{0.67}$ equation (Meeh, 1879) leads to estimates of body surface area that are markedly higher than those obtained with the other equations. Within a species, body mass increases during growth and aging. Increases in body weight are associated with changes in the proportion of body parts, primarily in the relative reduction of body appendages (Brody, 1945), which would reduce surface area per unit body weight. This feature of growth and aging can add precision to equations based on direct measurements of the surface area of animals of different weights within a species. Equations relating body mass to body surface area were developed based on measurements made on animals 50-60 years ago. The size of adult dairy animals has increased since then due to genetic and environmental changes; therefore, it is possible that the relationship between body surface area and body mass has been altered, which may reduce the applicability of these equations. The aim of this work was to study the relationship among the biometric measures of the sheep and the volume and surface and the body weight, to develop a physical model of sheep and to put the basis for a preliminary model of the heat stress response dynamics in small ruminants.

6.3. Matherial and methods

As described in Chatper 2, fifteen Sarda dairy ewes (3 years old), located in the experimental farm of the Department of Agriculture of University of Sassari located in the north-west of Sardinia (Sassari, Italy; 40°48’41.4” N 8°17’50.7” E) without cooling system to induce heat stress, were divided in three groups homogenous for body weight (48.2+-3.57 kg) and different for milk yield (MY): High MY, Low MY and Dry with 1900, 1200, 0 g/d of milk per ewe (n=5), respectively. Physiological variables trend was studied in this experimet as explained in the Chapter 2, but beyond this, other biometrical measures were registered. Through the combination of some of these parameters it was also possible to obtain the total body surface area, total body volume and their ratio.

Biometric measures

By approaching the concept of calculating the surface and body volume in a different way compared to what is reported in the various scientific publications, a geometric model (Figure 1a) was developed using biometric measurements for the calculation of these body variables (Figure 1b). To achieve this goal, two types of geometric solids were used: the cylinder, for the limbs; the truncated cone, for the neck, for the head and two for the torso. In the specific case of the torax, two truncated cones were used to represent respectively the anterior and posterior halves of the torso, being the widest abdominal circumference between the inguinal and thoracic ones. To calculate the surface and volume of the anterior truncated cone, the chest circumference (measured behind the forelimbs) was used for the smaller base and the circumference of the abdomen for the larger base; the height of the solid (h) was associated with half the length from the withers to the ileum. The formulas used are the classics of the geometry of solids:

- $S_l = \pi a(r + R)$
- $S_{ToT} = S_l + \pi r^2 + \pi R^2$
- $V = \frac{\pi h(r^2+rR+R^2)}{3}$
- $a = \sqrt{h^2 + (rR)^2}$

where “r” is the radius of the smaller circumference; “R” is the radius of the larger circumference and “a” is the apothem.

The same formulas described above were used to calculate the surface and volume of the posterior truncated cone; however, for the smaller base the circumference of the groin was used (taken in front of the hind limbs) and for the greater base the circumference of the abdomen was used, as for the anterior truncated cone. From the sum of the surfaces of the anterior and posterior truncated cone, the total surface of the trunk was obtained, the same for the total volume. The formulas seen above were used to derive the surface and volume of the neck and head. In the first case, for the smaller base was used the smaller circumference of the neck, for the greater base was used the greater circumference of the neck and the length of the neck up to the withers was considered as the apothem, from whose inverse formula has been obtained the height of the solid; in the second, for the smaller base was used the circumference of the muzzle at the height of the nostrils, for the greater base was used the circumference which considers the angle of the jaw with the perpendicular point of the nose and the length from the mental hole to the angle of the mandible was considered as the apothem from whose inverse formula was obtained the height of the solid. The cylinder formulas were used to calculate the area and volume of the limbs. In this case, the diameter of the cylinder, obtained from the circumference of the shin and from the circumference under the hock, respectively for the forelegs and hind limbs, was considered constant for the entire height of the cylinder (represented by the height at the chest and the height at the groin, for the forelegs and hind limbs respectively). The formulas used were:

- $S_l = 2\pi r \cdot h$
- $S_{ToT} = 2\pi r \cdot (h \cdot r)$
- $V = \pi r^2 \cdot h$

For the relationship between somatic measurements, volume and surface area of the animal, data analysis was performed with descriptive statistics and linear correlation techniques. For the analysis of physiological parameters as a function of time and environmental conditions, a mixed model was used with the PROC MIXED procedure of the SAS software (version 9.2. SAS Ins.). Physiological parameters such as respiratory rate, heart rate, oxygen saturation, rectal temperature, vaginal temperature, ear temperature, breast temperature were included as dependent variables. Among the independent variables, temperature classes (or THI) observed during the day were considered as fixed effects; time, day of the survey and the identification number of the individual animals (ID) as random effects; milk yield of each animal at the start of the test was included as a covariate. Differences were tested for significance at $P<0.05$ with Tukey’s test.

6.4. Results and discussions

*6.4.1. G*eometric model

In the first step of the work, a descriptive statistical analysis was carried out with the indication of the average, maximum values, minimum values, and standard deviation of the somatic measurements recorded in the barn. Based on the geometric model, the measurements of total body volume and surface area (with and without limbs), total body volume (with and without limbs) and their ratio were also estimated (Table 1). Through this procedure it was possible to have a complete picture of the distribution of the data in the sample compared to the average. Subsequently, a regression panel was performed between the animals' biometric measurements, weight, values of total surface area and total volume (both with and without limbs) and their ratio (Table 2). The relationships between the detected and estimated biometric measurements are shown in Table 1 and 2 and in the regression list in which the significant relationships observed between detected measurements and estimated body volume and surface area are shown in Table 3. From 1 to 12 equations are shown the significant regressions with $R^2 > 0.50$ explaining the most part of variability. The regression in which surface and volume vs. the circumference of the abdomen vs. $R^2 = 0.60$ and $R^2 = 0.66$ ($P<0.001$) were the most relevant ones (Figure 3 and 4).

Data from Chapter 2 and biometric relationships developed in the previous section allowed to develop two multiple regression equations considering animal response, air temperature and milk yield and biometric animal features from experiment described in Chapter 2:

- RR acts/min = - 145 + 8.19 AT (°C) + 0.00724 * MY (l/d) + 0.000757 * BV, cm^3 (Table 3) where RR is respiratory rate and AT, MY and BV represent barn air temperature, milk yield and body volume, respectively

- RR acts/min = - 41.5 + 8.21 AT (°C) + 0.00644 MY (l/d) - 250 S/V ratio (Table 3)

where RR is respiratory rate and AT, MY and S/V represent core temperature, milk yield and body surface area to body volume ratio, respectively.

The results from chapter 2 elaborated from this perspective showed that heat stress causes an increase in the animal's metabolic effort. The consequence of this is that an animal exposed to stress condition's takes a series of thermoregulation measures which have priority over the production objective and orient the metabolism towards a system aimed at obtaining a body thermal balance. The increase in respiration rate is just on of these mechanisms. The various levels of temperature observed during the experiment showed that the variability of the breathing rate is explained by more than 50% by the increase in ambient temperatures between 18 and 33 °C. For the purpose of an increasingly detailed understanding of the physiological responses of animals to weather variables it is possible to model the animal's thermoregulation mechanism. The geometric model and the relationships between physiological parameters and environmental temperature developed in this work can be used for future studies that allow the improvement of the estimate of the maintenance energy needs of sheep starting from somatic measurements and productive performances. The implications of these approaches are to improve sheep production performance, feed efficiency and animal welfare.

6.4.2. Conceptual modelling of heat stress

To explain the dynamics of heat stress and the trend of heat flows inside the animals, it’s been developed a causal loop diagram for explain the interactions of variables associated with heat stress in dairy sheep. The modeling techniques referred to the System Dynamics (SD) and System Thinking approach which is a method that extends beyond the conventional domain of the systems approach to large-scale complex problems. SD deals with the interaction of various elements of a system over time and captures the dynamic aspect by incorporating concepts such as stocks, flows, feedback, and delays, thus providing insight into the dynamic behavior of the system over time (Sterman, 2000). As a knowledge domain, SD can be thought of as a logical extension of systems engineering (SE) and systems analysis (SA). SD explicitly considers the dynamic behavior that results due to delays and feedback in the system. SD has had a significant intellectual impact around the world. System dynamics as a method has been successfully applied in a wide variety of business and socioeconomic sectors to understand problems and gain insight into various events (Tang and Samudra, 2001).

This developed causal loop diagram or CLD is composed by a set of variables connected by arrows denoting the causal influence. The flows always start from independent variable to dependent variable with causality links identified by the arrows. Assigned to each arrow there is a polarity sign (+/-) indicating the statistical correlation between variables. With positive sign (+) the two variables considered move in the same direction; with negative sign (-) variables move in the opposite direction. Inside this diagram there are also two typologies of loops: reenforcing loops (R) indicates that the system promotes the growth or decline of the same; on the contrary balancing loops (B) indicates that the system promotes the regulation of the same (Sterman, 2000). In this case are utilized variables to explain animals physiological, behavioural and productive responses relatively to environmental conditions following the example of Molina-Benavides et al. (2018). In summary the diagram traces the animal exposition to heat environments influencing body heat production (reinforcing loop R+ derivating from genetic stimulus to produce milk and increase feed intake) and dissipation (balancing loop; B-) that involves all the dissipation physiolòigical mechanisms. With the increase of body heat stock, the animal start a complex system of defence, initially with the decrease of the feeding intake (that determines a risk of negative energy balance) and with the increase of water intake. This latter influence most part of the physiological responses of the animal to contrast the discomfort. Physiological responses includes sweating, panting, respiratory rate, pulse rate, skin temperature and rectal temperature as reported in the CLD (Figure 2).

6.5. Conclusions

This work adds a little piece of information to further model the relationships among animal body weight, surface and volume and heat stress. It is important to consider that animal body surface and volume can be estimated trough biometric measures or with technological tools that take into account animal images from barn. All these info need to be used to develop a dynamics model of heat stress response in sheep. A conceptual diagram of the factors affecting animal response to heat stress is also added to this chapter.

6.6. References

Berman, A. 2003. Effects of body surface area estimates on predicted energy requirements and heat stress. J. Dairy Sci. 86, 3605–3610. https://doi.org/10.3168/jds.S0022-0302(03)73966-6

Gebremedhin, K.G., Wu, B. 2001. A model of evaporative cooling of wet skin surface and fur layer. J. Therm. Biol. 26, 537–545. https://doi.org/10.1016/S0306-4565(00)00048-6

Johnson, H.D. 1987. Bioclimates and Livestock. In Bioclimatology and the Adaptation of Livestock (ed. Johnson, H.D.), Elsevier Science Publishers, Amesterdam, pp. 3–16.

Marai, I.F.M., Shalaby, T.H., Bahgat, L.B., Abdel-Hafez, M.A. 1997. Fattening of lambs on concentrates mixture diet alone without roughages or with addition of natural clay under subtropical conditions of Egypt. Growth performance and behaviour. In: Proceedings of International Conference on Animal Production & Health, Dokki, Cairo, Egypt.

McGovern, R.E. and Bruce, J.M. 2000. A model of the thermal balance for cattle in hot conditions. J. Agric. Eng. Res. 77(1), 81–92.

Molina-Benavides, R.A., Gurrero, H.S., Atzori, A.S 2018. A conceptual model to describe heat stress in dairy cows from actual to questionable loops. Acta Agron. 67(1), 59–64. https://doi.org/10.15446/acag.v67n1.60612

Pennisi, P., Costa, A., Biondi, L., Avondo, M., Piccione, G. 2004. Influence of the fleece on thermal homeostasis and on body condition in Comisana ewe lambs. Anim. Res. 53, 13–19. https://doi.org/10.1051/animres:2003047

Sterman, J.D. 2000. Business Dynamics, Systems Thinking and Modeling for a Complex World. Mc Graw-Hill (Eds.). Boston, MA, USA. p 982

Tang, V. and Samudra, V. 2001. System Dynamics. Origins, development, and future prospects of a method. Research Seminar in Engineering Systems. ESD. 83.

Thompson, G.E. 1985. Respiratory system. In Stress Physiology in Livestock (ed. Young, M.K.), CRC Press, Inc., Boca Raton, Horida, USA, pp. 155–162.

Turnpenny, J.R., Wathes, C.M., Clark, J.A., McArthur, A.J. 2000. Thermal balance of livestock: 2. Applications of a parsimonious model. Agric. For. Meteorol. 101(1), 29–52. https://doi.org/10.1016/S0168-1923(99)00157-4.

6.7. Tables and Figures

Table 1. Descriptive statistics with mean, standard deviation, maximum and minimum of the somatic measurements, surfaces, volumes and body weight of 15 Sarda dairy sheep.

	Unit	Average	±SEM	Max	Min
Withers height	cm	64.57	2.91	72	60
Croup height	cm	68.03	3.91	78.5	63.5
Chest height	cm	34.42	1.22	36.5	33
Inguinal height	cm	34.13	2.05	39.5	30
Chest circum.	cm	86.13	3.87	94	80
Abdomen circum.	cm	105.67	5.58	116	95
Inguinal circum.	cm	96.13	3.87	104	90
Hock circum.	cm	12.20	1.28	14	10
Shin circum.	cm	9.39	0.47	10	9
Min. Neck circum	cm	29.40	2.41	37	27
Max. Neck circum.	cm	31.87	2.29	38	28
Ischia lenght	cm	14.71	0.55	15.5	13.5
Ileus lenght	cm	18.08	0.94	20	17
Neck-Withers lenght	cm	34.60	3.07	40	29
Withers-Ileus lenght	cm	46.33	2.23	50	42
Ears-Tail lenght	cm	101.93	4.48	112	93
Body surface	m^2	1.15	0.07	1.28	1.02
Body volume	m^3	0.04	0.004	0.05	0,034
Body surface (without limbs)	m^2	0.89	0.055	0.98	0.81
Body volume (without limbs)	m^3	0.04	0.004	0.046	0,033
Body weight	kg	45.40	4.15	51.30	39.10

Table 2. Regressions between biometric measures and total body surface area (with and without limbs), total body volume (with and without limbs), and body weight.

	TS		**TV**		**TS_WL**		**TV_WL**		**BW**	
	r	**p-value**	**r**	**p-value**	**r**	**p-value**	**r**	**p-value**	**r**	**p-value**
Withers H	0.45	NS	0.42	NS	0.39	NS	0.41	NS	0.18	NS
Croup H	0.60	*	0.62	*	0.58	*	0.61	*	0.45	NS
Chest H	0.05	NS	0.18	NS	0.18	NS	0.20	NS	0.42	NS
Inguinal H	0.11	NS	0.15	NS	0.12	NS	0.17	NS	0.36	NS
Chest C.	0.52	*	0.67	**	0.72	**	0.68	**	0.76	***
Abdomen C.	0.72	**	0.81	***	0.77	***	0.81	***	0.76	***
Inguinal C.	0.73	**	0.68	**	0.68	**	0.67	**	0.46	NS
Hock C	0.56	*	0.14	NS	0.10	NS	0.09	NS	0.02	NS
Shin C	0.34	NS	0.37	NS	0.34	NS	0.37	NS	0.42	NS
Min. Neck C	0.13	NS	0.04	NS	0.03	NS	0.04	NS	0.02	NS
Max. Neck C	0.24	NS	0.14	NS	0.21	NS	0.14	NS	0.23	NS
Ischia L	0.38	NS	0.53	*	0.56	*	0.54	*	0.55	*
Ileus L	0.52	*	0.71	**	0.75	***	0.72	**	0.70	**
Neck-Withers L	0.16	NS	0.33	NS	0.03	NS	0.33	NS	0.12	NS
Withers-Ileus L	0.45	NS	0.67	**	0.65	**	0.69	**	0.50	0.056
Ears-Tail L	0.27	NS	0.36	NS	0.36	NS	0.36	NS	0.30	NS
Body weight	0.69	**	0.87	***	0.88	***	0.87	***	-	-

*p<0.05, **p<0.01, ***p<0.001; C=circumference; H=height; L=lenght

Table 3. Regression equtions among volume or surface and biometric measures and among respiration rate and bbiometric measures.

Y	Intercept	Coef. 1	Variable 1	Coef. 2	Variable 2	Coef. 3	Variable 3	R2	P
BS-WL	93.14	102.4	Chest C.					0.52	0.01
BV-WL	19402	672.34	Chest C.					0.47	0.01
BS-WL	873	76	Abdomen C.					0.60	0.01
BV-WL	19743	551	Abdomen C.					0.65	0.01
BS	1152	132	Inguinal C.					0.53	0.01
BV	25494	679	Inguinal C.					0,47	0.01
BS	1947	91	Abdomen C.					0,53	0.01
BV	19206	556	Abdomen C.					0,67	0.01
BW	13.4	0.56	Abdomen C.					0.56	0.01
BW	25,5	0,82	Chest C.					0.59	0.01
BS-WL	1010	437	Ileus L					0,56	0.01
BV-WL	14015	2905	Ileus L					0.52	0.01
RR	42.82	8.625	AT	0.008	MY	250	S/V	0.58	0.01
RR	-145	9.19	AT	0.007	MY	0.0008	BV	0.55	0.01

*all the regressions are significant for $p<0.05$; AT=air temperature; MY=milk yield; BS=body surface; BV=body volume; BS-WL=body surface whitout limbs; BV-WL=body volume whitout limbs; B/V = surface volume ratio from biometric measures (Figure 1).

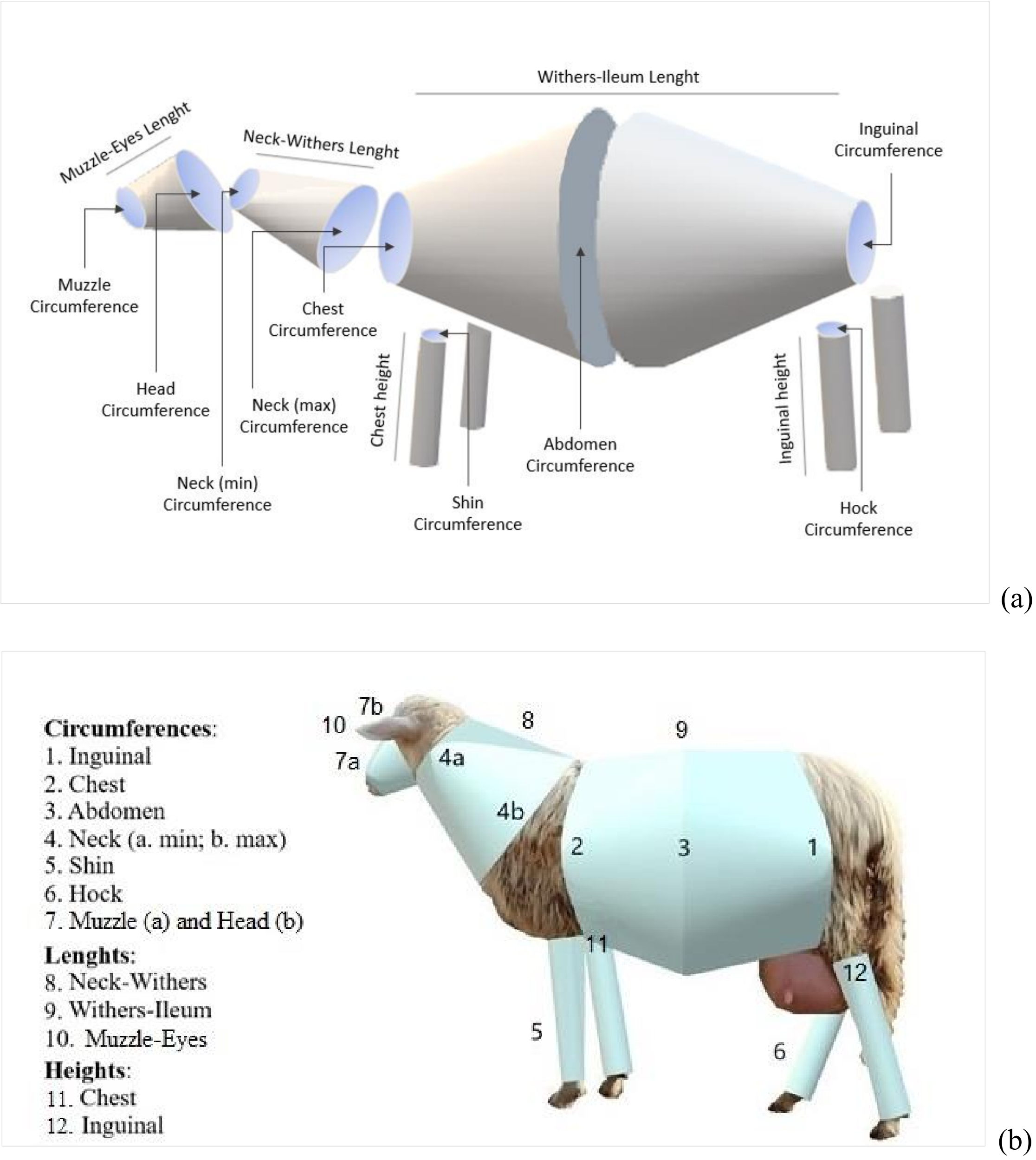


Figure 1. Descriptive list of the biometric measures of the sheep used for the phisic model in the conceptual (a) and sheep adapted versions (b).

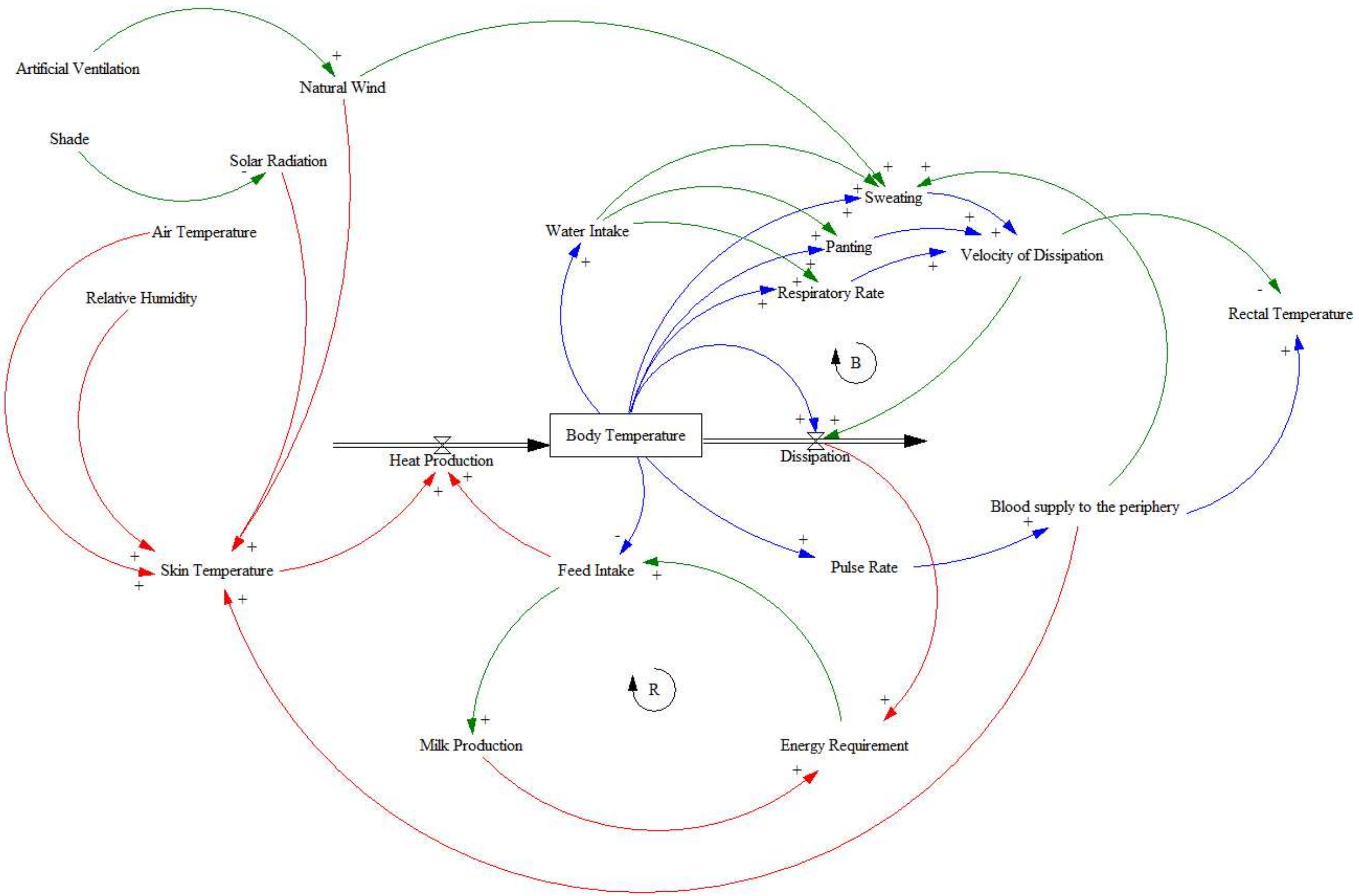


Figure 2. Causal loop diagram of a preliminary heat stress model fro small ruminants.

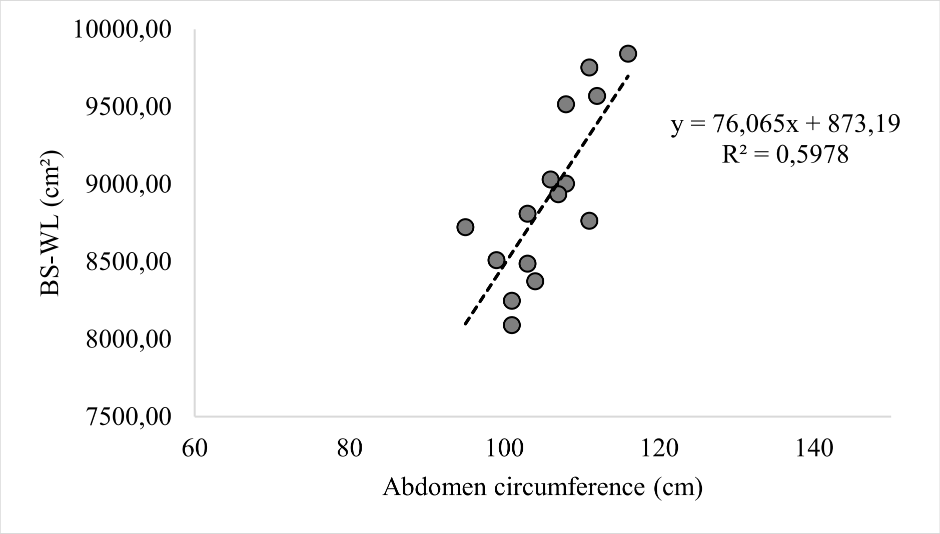


Figure 3. Regression between abdomen circumference (cm) and body surface whitout limbs (BS-WL; cm2).

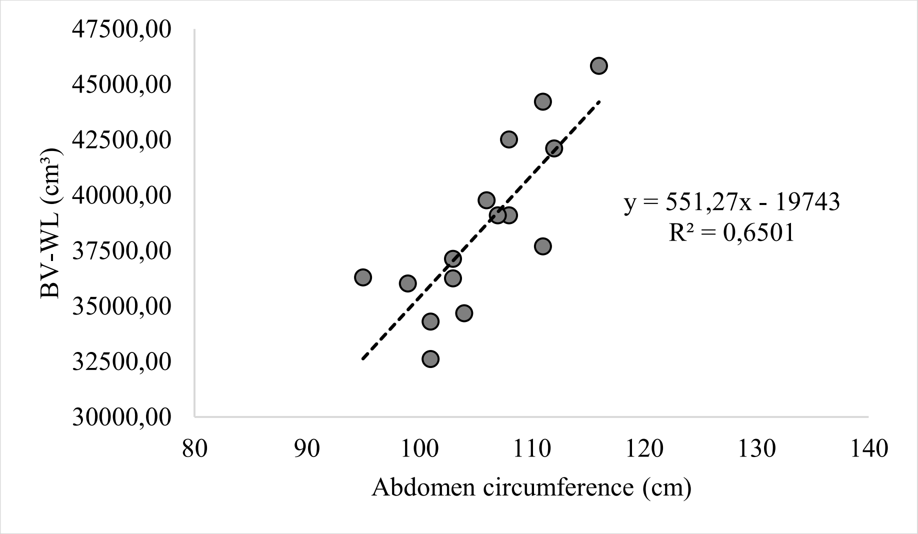


Figure 4. Regression between abdomen circumference (cm) and body volume whitout limbs (BV-WL; cm^2).

CHAPTER 7

General conclusions

7. GENERAL CONCLUSIONS

Sheep are considered one the most favoured species to adapt to climate change in the next decades. Nevertheless, they suffer heat stress intensively. Several aspects of understanding animal response to heat stress need to be carried out. In particular: i) biological markers of heat stress need to be defined at field level to estimate and quantify the biologic effort related to heat stress in sheep; ii) calorimetric measures of energy expenditure for heat dissipation and under heat stress conditions need to be carried out in order estimate the maintenance adjustments for energy requirements related to heat stress.

This Dissertation contributes markedly to the first point. In particular presents a broad study on the main parameters that characterize the animal response to heat stress in Sarda dairy sheep. In particular the respiration rate have been deeply studied in any conditions and in term of daily and hourly variation in sheep housed indoor, dry and lactating. Respiration rate has been considered the most important variable to be estimated in order to model heat stress and as a proxy of heat stress in Sarda dairy sheep.

Relatively to second point the dissertation contributes with a preliminary model and with specific equations relative to the surface/volume ratio in Sarda dairy sheep. It allows to put the basis for the auxiliary constant rates that have to be take into account in a mechanistic heat stress model for small ruminants. Table 1, reporte the equations for dairy sheep presented in the various chapters that specifically describe the variability of respiration rate as marker of heat stress in Sarda dairy sheep. It brings valuable knowledge for the specific application in Mediterranean areas where the breed is diffuse for milk production.

About the third point, the work of Thesis provides no information about calorimetric measures at this stage. Nevertheless, it provides two important datasets, developed from studies in metabolic cages and ventilated hoods equipment's for indirect calorimetry. Those data can be elaborated to start the calorimetric quantification of maintenance requirements estimation under heat stress conditions. Furthermore an estimation of the interaction among heat stress and methane emissions have been quantified in ventilated hoods equipments.

This work presents a focus on the variables but does not include any indication about innovative technology, tool or digitalization techniques that could be used at farm level to measure the animal performaces. Otherwise data from this thesis can be used at farm level as reference of animal response and could be useful to develop specific tools aimed at estimating the respiration rate of ewes.

Future studies need to be focused to i) study the effect of ventilation and cooling systems on the estimated parameters (i.e.: respiration rate and body core temperatures); ii) to deeply work on the development of a dynamic model of heat stress in order to improve the estimation of dietary calculations and the precision feeding of small ruminants in indoor systems.

7.1. Tables

Table 1. Proposed equations for dairy sheep as presented in the various chapters.

Chapter	Category	Equation	R^2
2	Dry	RR = 8.0478 * AT (° C) - 105.23	0.58
2	Dry	RR = 4.52 * THI (°F) - 209.66	0.26
3	Dry	RR = 40.7 + 0.00246 AT (° C) - 12.8 Season + 2.72 S/V	0.58
4	Dry	$RR = 0.2555^{\wedge 0.0739*THI}$	0.40
4	Dry	RT = 1.2867 + 1.0199 * PT (°C)	0.90
5	Lactating	CH_4 (g/d) = 2.2452 * THI (°F) - 151.44	0.40
5	Lactating	WI (g/d) = 3.3873 * DMI (g/d) + 0.2224	0.45
6	Lactating	RR = - 145 + 8.19 AT (°C) + 0.00724 *MY (l/d) + 0.000757 * BV	0.55
6	Lactating	RR = - 41.5 + 8.21 AT (°C) + 0.00644 * MY (l/d) – 250 * S/V	0.58

RR=respiratory rate (acts/min); RT=rectal temperature (°C); WI=water intake; AT=air temperature; S/V=body surface and volume ratio; PT=ocular globe; BV=body volume; DMI=dry matter intake

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