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RESPONSE TO CLIMATE CHANGE IN THE SHEEP PRODUCTION SYSTEM:
ADAPTABILITY TO WATER AND HEAT STRESS

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INDEX

ABSTRACT

This thesis examines the impacts of climate change on animal welfare, with a particular focus on heat stress and the decreasing availability of water. The research considered a sample of 40 sheep, monitored through measurements to assess the influence of these environmental conditions on weight trends, Body Condition Score (BCS), and the intake of food and water. The results highlight a significant correlation between the rise in temperatures and changes in feeding behaviour and the productive efficiency of the animals. Notably, a tendency towards reduced weight and BCS under thermal stress conditions is observed, coupled with an increased water consumption. These data underscore the importance of adaptation and improvement strategies to preserve animal welfare in the context of climate change, with direct implications for the sustainability of farming practices. Furthermore, the thesis proposes guidelines for managing sheep farms in at-risk environments, emphasizing the need for a multidisciplinary approach that includes environmental, ethological, and physiological considerations.

RIASSUNTO

Questa tesi esamina gli impatti dei cambiamenti climatici sul benessere degli animali, con particolare attenzione allo stress da calore e alla minore disponibilità di acqua. La ricerca ha preso in considerazione un campione di 40 pecore, monitorato attraverso misurazioni per valutare l'influenza di queste condizioni ambientali sulle tendenze di peso, Body Condition Score (BCS), e l'assunzione di cibo e acqua. I risultati evidenziano una significativa correlazione tra l'aumento delle temperature e i cambiamenti nel comportamento alimentare e l'efficienza produttiva degli animali. In particolare, si osserva una tendenza verso un peso ridotto e BCS in condizioni di stress termico, insieme ad un aumento del consumo di acqua. Questi dati sottolineano l'importanza di strategie di adattamento e miglioramento per preservare il benessere degli animali nel contesto del cambiamento climatico, con implicazioni dirette per la sostenibilità delle pratiche agricole. Inoltre, la tesi propone linee guida per la gestione degli allevamenti ovini in ambienti a rischio, sottolineando la necessità di un approccio multidisciplinare che comprenda considerazioni ambientali, etologiche e fisiologiche.

1.INTRODUCTION

1.1 Climate change and the impact of heat stress on small ruminants

The documentation of how some of the anticipated climate changes could concretely affect the forthcoming availability of ruminant animal products is clear. In particular, it is the increase in atmospheric levels of carbon dioxide, the rise in temperature, the variations in quantity, seasonality and variability of rainfall and the manifestations of increasingly extreme weather events. The consequences of climate change, specifically the increase of temperature, on farming systems are directly perceptible in relation to the physiological aspect, behavioural, productive and animal welfare, and indirectly to the quantity, quality and the availability of feed (Henry et al., 2018).

Compared to the previous century, the Earth's annual average temperature has risen by 0.94°C, representing a certainly concerning rate of increase. An essential factor in assessing the severity of this situation pertains to, as anticipated, rainfall conditions, their intensity, and seasonality. What becomes evident is that the spatial models designed for future resilience are already present and measurable, providing us with the opportunity to analyze and evaluate immediate adaptive responses, as well as long-term provisions.

Efficiency in adapting over time to worsening climatic conditions will be closely tied to the type of livestock management one wishes to practice. An observation, among others, has shown, for example, that the milk production of Holstein-Jersey cattle in tropical or subtropical conditions can be up to 40%-60% lower compared to mild climate conditions. This underscores the severity of the situation but also highlights the importance of directing livestock strategies towards adaptability rather than mere productivity. The most effective adaptation options will combine technological, behavioral, managerial, and policy alternatives to efficiently offset the negative effects of climate change and maximize benefits.

When considering climate change and new management technologies in reference to ruminants, let’s analyze the cases of cattle and sheep more closely, as we have more data and research in this regard, thanks to the response of breeders concerning practical and strategic adaptability to the anticipated climate changes (Henry et al.,2018).

Small ruminants have always been a significant productive resource for small-scale livestock farmers, especially in arid regions where we find approximately 56% of the domestic ruminant population. This is because this category of animals possesses a high level of resilience, even in the presence of stress factors. Consequently, the study of their genetics and physiology can bring researchers closer to continually refining practices and adaptation strategies. It has been estimated that the risk of extreme heat stress is real and will not only affect rising temperatures but also the emergence of pathogens and diseases, as well as the quantity and quality of the diet (McManus et al., 2022).

The values to monitor as warning signs are air temperature, relative humidity, wind speed, and solar radiation intensity. Heat stress is termed acute when the temperature change occurs suddenly, and if the condition persists over time, it becomes chronic. In the presence of heat stress, animals attempt to restore their homeostasis through the mobilization of water and energy, reduction in productive and reproductive activities, and decreased appetite (McManus et al., 2022). Immune activity also comes into play in achieving this goal, and in the case of significant alterations, it may become more susceptible to diseases and antigens. However, when the stress factor is mild, the adaptive immune response can be beneficial to the organism as it leads to an increase in cellular defenses, followed by an enhancement of physical performance (McManus et al., 2022).

In particular, heat stress refers to a phenomenon closely related to a more or less significant increase in external temperature. The "stressful" connotation arises from the fact that this temperature variation affects the normal physical, physiological, and behavioral conditions of animals, to a greater or lesser extent depending on the species and breed. Heat stress results not only from the temperature itself but also from the combination of multiple factors such as humidity, solar radiation, wind speed, and the animal's ability to regulate its body temperature (Herbut et al.,2018).

The consequences that an animal affected by heat stress may experience include reduced productivity, decreased fertility, an increased susceptibility to diseases, and in severe cases, an elevated mortality rate. There is no specific threshold beyond which these conditions begin to occur; it depends on the intrinsic characteristics of the animal, including species, age, breed, genetic potential, size, and previous exposure.

A significant factor contributing to the progressive increase in the discussed issue is undoubtedly anthropogenic activities, especially greenhouse gas emissions. In some countries already affected by a tropical or subtropical climate, such as Brazil, the average daily weight gain in pigs has decreased by

approximately 10%, along with a reduction in food intake of about 14%, compared to regions with a milder climate (Thornton et al., 2021).

Those who handle heat stress better compared to the issues raised are sheep and goats, animals with significant rustic capabilities that allow them to adapt relatively quickly, thus limiting risk factors. Goats, even more than sheep, fall into this category.

There are various methodologies for assessing the level of heat stress, most of which involve the use of indices. Often, the goal is to correlate, through the use of these indices, the level of discomfort imposed on animals at a given temperature and the actual temperature perceived by the animal itself (Herbut et al., 2018). The most widely used index is the Temperature-Humidity Index (THI), and the equation commonly used in this regard is that of Thom (1959):

$$THI = 0.8 \times T + ((RH/100) \times (T - 14.3)) + 46.4 \quad (1)$$

Where T is the dry bulb air temperature (°C), and RH is the relative air humidity (%). The equivalent equation provided by the National Research Council (1971) is:

$$THI = (1.8 \times T + 32) - (0.55 - 0.0055 \times RH) \times (1.8 \times T - 26.8) \quad (2)$$

The THI humidity index has been widely used in the literature to establish threshold limits within which the animal will not yet exhibit physiological changes due to climate alterations, such as changes in respiratory rate and body temperature. Studies have shown, for example, that dairy cows are more susceptible than beef cattle, experiencing a loss of production with THI values above 72, compared to the 78.2 of beef cattle (Thornton et al., 2021).

To conduct global studies on different THI tolerance, formulas (1) and (2) have been used to determine additional ranges beyond those previously mentioned, within which to place the most relevant livestock species. Sometimes differences have been identified even within intraspecific breeds, as in the case of 12 breeds of sheep from Europe and Australasia.

Table 1: Selected studies using the Thom (1959) equation for the temperature humidity index (THI) to estimate heat stress in animals.

Species	Onset of heat stress level			References
	Moderate	High	Extreme	
General	70	75	80	Thom (1959)
General	72	78	90	Fuquay (1981)
Cattle—dairy	72	79	89	Moran (2005); Dunn et al. (2014); Ranjitkar et al. (2020); Rahimi et al. (2021); Pinto et al. (2020)
Cattle—general	72	79	90	Xin & Harmon (1998)
Cattle—beef	72	82	94	Valente et al. (2015)
Goats	70	79	89	Serradilla et al. (2018)
Sheep	72	78	90	McManus et al. (2016); Beldadj Slimen et al. (2019)
Pigs	75	79	84	Xin & Harmon (1998); Lallo et al. (2018); Mutua et al. (2020)
Poultry—broilers	74	79	84	Oliveira et al. (2019)
Poultry—layers	71	76	82	Du et al. (2020); Xin and Harmon (1998)
Poultry—general	73	81	85	Moraes et al. (2008)

Source: Increases in extreme heat stress in domesticated livestock species during the twenty-first century (Thornton et al.,2021).

1.2 Influence of heat stress in sheep

As for many species, in sheep, the counteraction of excessive ambient heat occurs through the dissipation of body heat into the environment. In this case, the animal releases part of its thermal load into the environment through the evaporation of respiratory tract water and from the superficial part of the skin through sweating and hyperventilation. In the case of sheep, the woolen coat limits sweating, making it less effective in dissipation. Therefore, with an increase in temperature to 36°C, most of the heat is released through the legs and ears (Marai et al., 2007).

When these physiological mechanisms are not sufficiently effective in countering environmental heat stress, the rectal temperature increases. As a result, there are biological anomalies due to prolonged exposure to excessive temperatures, such as imbalances in water, protein, energy, and mineral balances, decreased food intake, hormonal and metabolic secretions in the blood. Another example of biological dysfunction due to high temperatures involves the suprachiasmatic nucleus (SCN), a hypothalamic nucleus responsible for regulating the animal's circadian and seasonal cycles. The SCN acts through the phasic and tonic release of hormones, so a malfunction in its operation can lead to a significant reduction in the animal's productive and reproductive capacity (Marai et al.,2007).

Regarding reproduction, in tropical and subtropical areas, sheep mainly reproduce in the summer months, and high temperatures are a major impediment to the species' prolificacy in such regions. This effect worsens further if heat stress is accompanied by high humidity. In the early stage of gestation, the embryonic phase, there is a presence of significantly limited placentomes, a condition that may slightly worsen following exposure to high temperatures (30-40°C, 40% humidity) compared to thermoneutral temperatures (18-20°C, 30% humidity) .

All of this is deduced from an examination of the protein and energy metabolism of the placenta towards the middle and end of gestation. Specifically, the ratio of RNA to DNA indicates the placental protein synthesis capacity, which, under heat stress conditions, is reduced, resulting in a reduction in fetal development, especially in the first 17 days of pregnancy. In conclusion, the birth weight of lambs of sheep breeds is indirectly proportional to the exposure of mothers to hot or semi-arid environments; the greater the exposure over maternal generations, the lower the birth weight. It has been inferred that the presence of shaded areas is important to improve the pregnancy rate in the flock (Marai et al.,2007).

If we analyze the productive aspect, we see that the first symptoms of excessive temperature are a decrease in body weight, which, in turn, implies a reduction in average daily gain (ADG), a decrease in the growth rate, and, in general, a lower appreciation of the live weight of the animal, resulting in an economic loss.

1.2.1 Animal adaptation techniques to heat stress

As previously introduced, heat stress is a phenomenon that fundamentally affects every aspect of livestock life, from productive and reproductive characteristics to physiological and behavioral aspects. The adaptation of the latter to temperature variation is the first and primary attempt by the animal to reduce the heat load (Seijan et al.,2018).

One of the most rapid changes when it comes to heat stress is seeking shade, as animals try to escape direct heat load. Another adaptation signal that ruminants manifest concerns their relationship with feeding; initially, a reduction in appetite will be noticed as the animal tries to counter the external temperature by regulating internal metabolic heat production (Seijan et al.,2018). Secondly, nutritional activity will be concentrated during the cooler hours of the day, early in the morning and later in the evening. If attention is also paid to posture, it can be observed that animals tend to spend more time standing, allowing them to orient themselves according to the incidence of sunlight, avoiding direct radiation whenever possible. Furthermore, by standing, the area of skin exposed to airflow or wind is greater than in a lying position, facilitating the dissipation of heat into the external environment (Seijan et al.,2018).

The quantity and frequency of water intake are also influenced by the reduction in urine production when water is scarce or by an increase in water consumption to counter tissue evapotranspiration (McManus et al., 2022). Evapotranspiration is indeed one of the primary mechanisms employed by the animal's body to lower body heat in case of heat stress; up to about 80% of total evaporative heat loss can be dissipated through the skin. The water evapotranspiration capacity is associated with a vapor pressure gradient, which, in the presence of high humidity, reduces, thus lowering the rate of heat loss from tissues (Barnes et al., 2004).

This phenomenon is often associated with an increase in respiratory frequency. For example, under severe heat stress conditions, sheep's respiratory frequency can reach 300 breaths per minute. Therefore, the perceived stress level of the animal can be categorized by referring to the breathing rate: low heat stress: 40–60 breaths per minute, moderate heat stress: 60–80 breaths per minute, high heat stress: 80–200 breaths per minute, severe heat stress: over 200 breaths per minute (Gougoulis et al., 2010).

Respiratory rate increases with rising external temperature, while the tidal volume, i.e., the amount of air the animal mobilizes with each breath, decreases. This dynamic, when not in critical conditions such as "high" or "severe" heat stress, allows heat dissipation while maintaining alveolar ventilation and thus the total gas exchange in the organism unchanged. The threshold beyond which the increase in respiratory frequency joins other mechanisms to counter excessive body heat depends on various factors such as humidity, ventilation, and air flow.

Another element involved in restoring normothermia is the metabolic rate. The organism undergoing heat stress tends to reduce daily metabolic activities according to their normal course, such as the progressive loss of appetite or a reduction in thyroid action, which also contributes to the decrease in the overall daily metabolic rate. Other hormonal responses include an increase in plasma cortisol following short-term exposure or a reduced cortisol turnover rate and a decrease in plasma concentration in the case of prolonged exposures (Barnes et al., 2004).

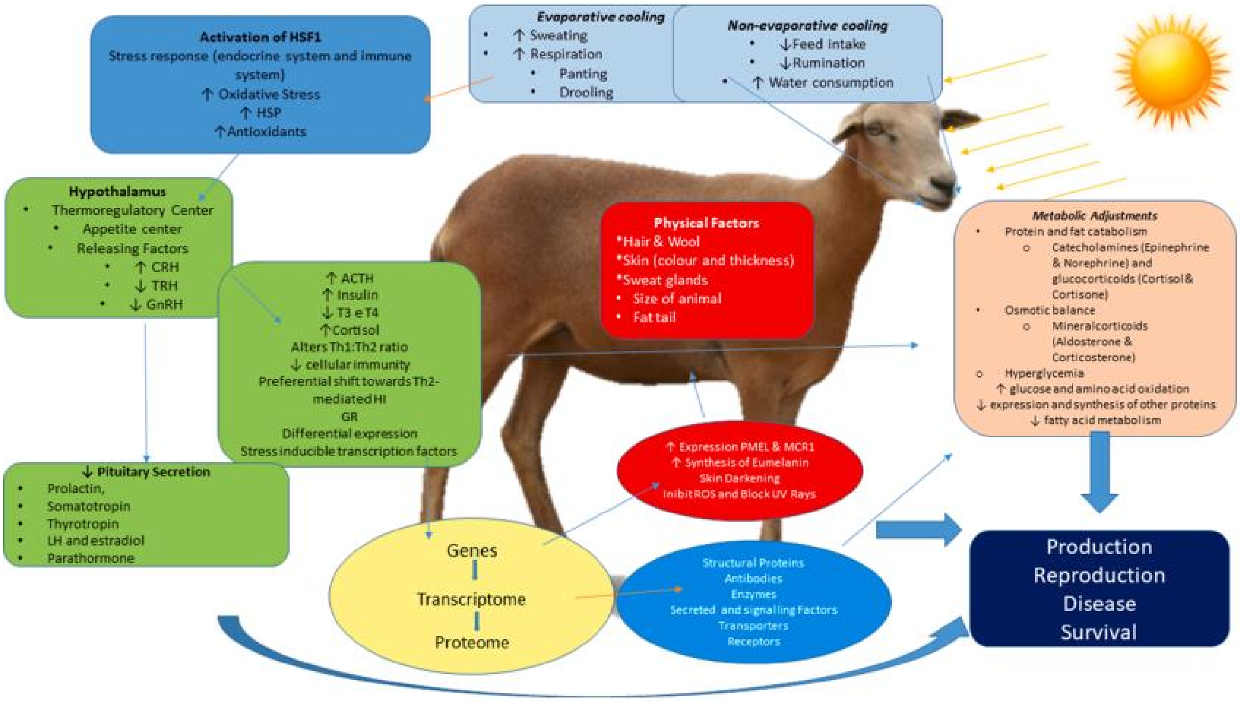
Let's now see how the phenotypic aspect of the animal also affects its ability to cope with heat stress. The first aspect to consider is the color of the coat; sheep with a light-colored coat have a higher reflective capacity (from 50% to 60%) and are therefore more suitable for facing direct radiation (Seijan et al.,2018). The skin pigmentation, the higher it is, provides more protection to the tissues. In fact, together with a shorter coat length and thin skin, it constitutes the main indicators of a good ability to adapt to more arid conditions. The fat tail observed in sheep is also recognized as a morphological adaptation for better heat transfer outside (Sejian et al.,2018). Another aspect that may be related to prolonged exposure to heat stress is the reduction in breed size.

Another "side effect" that could be connected to prolonged exposure to high temperatures is the reduction in breed size. In fact, the gradual birth of smaller individuals, for example, can be identified as a form of adaptation to the gradual increase in temperature. This is because exposure to high temperatures during the early development of the placenta compromises its proper growth due to slowed cell reproduction (Kandermir et al., 2013).

As a result, there might be an issue in early fetal development as well. However, it has been reported that the most significant negative effects are observed in the first 17 days of pregnancy, during which it is important to provide pregnant animals with shade and cooling (Kandemir et al., 2012). This condition worsens if associated with reduced energy availability and poor food quality, along with a decrease in the intake of essential nutrients. These factors, firstly, lead to a decrease in anabolic activity and an increase in tissue catabolism, which, in addition to accelerating the aforementioned process, over time trigger a physiological and hormonal response.

It has, therefore, been inferred that smaller breeds and/or those with longer and thinner appendages, such as those characterized by a white fur coat and darker skin, are better able to resist heat stress compared to other breeds of larger and shorter-boned individuals, despite the higher vulnerability to solar radiation (McManus et al., 2020).

Fig 1: Pathways of reaction to heat stress in small ruminants



Source: Response to heat stress for small ruminants: Physiological and genetic aspects (McManus et al., 2022).

1.3 Availability of feed in heat stress conditions

In arid environments, the management of small ruminants offers a significant advantage over larger species, allowing more animals per unit of land. Goats and sheep, being ruminants, rely mainly on forage as their main source of food. During the dry season, however, the supply of forage decreases and the available one becomes fibrous and lacking in proteins.

It is now well known that the drop in the quantity and quality of pastures and the decrease in the production of fodder crops is a clear consequence of the increase in temperatures and droughts. A change in seasonal patterns, caused by warmer and drier climates, results in significant yield reductions and alterations in the time pattern of grass production and grazing systems. Rising temperatures reduce the production of fresh grass and lead to lower quality and variety of forage species. Similarly, higher temperatures are associated with an increase in lignification in plant tissues and a consequent decrease in forage digestibility, directly influencing the availability of the latter for a balanced intake in the diet of animals. A lower quality of fodder means that the animals themselves may not meet the energy needs needed to maintain their body weight (Escarcha et al., 2018).

Cereal crop resources, on the other hand, consisting mainly of stems and leaf material, represent an essential food resource for livestock farmers during the dry season. Although they are rich in fiber and poor in crude proteins, it is necessary that these residues undergo a ruminal decomposition, a crucial process dependent on the presence of an adequate amount of cellulolytic bacteria. The rate of decomposition and the passage through the rumen are critical factors influencing forage intake.

Improving the nutritional value of these residues often requires an increased intake of nitrogen in the rumen, thus improving the digestibility of the fibers. The efficient use of crop residues has been extensively explored by various authors. To ensure maximum conservation of the nutrients contained, it is essential to begin storage at the collection stage. The timely harvest, as soon as the crop is sufficiently dry for storage after the grain harvest, reduces leaf losses between the field and storage, ensuring a higher nutritional value.

Dry storage is necessary to prevent mold formation, which could cause digestive problems in animals. The forage storage facility, constructed from locally available materials, should have a raised floor to prevent rising humidity and facilitate rodent control. A covered structure protects against damage caused by rain, while ensuring adequate ventilation. In summary, efficient management of crop residues requires care from the harvesting stage to maximize their nutritional value for livestock (Ben Salem et al., 2008).

2. WATER RESTRICTION

2.1 Water restriction and consequences on animal organism

In the regions of the Middle East and North Africa, small ruminants are mainly reared in traditional and extensive pastoral agricultural systems, depending on natural pastures and water and food patterns throughout the year. Precisely because of this strong interdependence, the irregular availability of rainfall and the scarcity of water are considered crucial issues in animal production in these areas. The growing concern for water scarcity has stimulated the search for solutions for its rational and sustainable use. An innovative management of this problem is even more urgent since water is also intrinsically linked to all biochemical processes and promotes the homeostasis of the organism and this means that it is a basic element for a good level of well-being animal (Araùjo et al.,2022).

In detail, the water requirements of small ruminants in temperate climates vary according to breed and adaptability, with goats requiring from 3.15 kg/kg dry matter (23 µc) to 4.71 kg/kg dry matter (35 µc). Sheep breeds show different capacities to cope with water limitations; for example, Yankasa sheep survive 5 days of water restriction, while the Awassi can withstand more than a month of drinking every 2 days without significant impacts on their health. Similarly, Australian Merino sheep survive up to 10 days without water, while the Bighorn desert sheep endure up to 15 days, while for example the Barki sheep in Egypt cannot stand more than 3 days without drinking.

The behavioural, morphological and physiological changes that small indigenous ruminants are able to adopt allow them to thrive despite extreme temperatures and limited water resources, in fact also the aspects related to production capacity are adaptable to this type of conditions. For this reason, we will analyse these modes of adaptation to understand the extent to which they make the animal resilient (Chedid et al., 2014).

This peculiarity is also relevant in semi-arid regions such as Brazil, where Santa Inês sheep are exploited for the production of meat in an extensive system, with little or no technology. The Santa Inês breed is considered promising for its adaptability, reproductive efficiency and low susceptibility to pests, playing an important role in protein production in areas characterized by a dry climate.

Water also has a significant impact on the chemical composition of meat, and water restriction can affect the quality of meat as a commercial product. The need for studies evaluating the effects of daily water supply reductions on meat quality, mineral composition and fatty acid profile in Santa Inês sheep is increasingly pressing. The main objective is to understand how and at what level, the water restriction affects the adaptability and resilience of small ruminants and their eating behaviour (Araùjo et al.,2022).

Behavioural adaptation:

The feeding and reproductive behaviour of small ruminants is strongly influenced by environmental conditions. For example, both Rocky Mountain sheep and goats exhibit nocturnal feeding behavior to avoid high temperatures during the day. In some adapted breeds, such as pigmy goats, a change in the frequency of meals is observed, which become more frequent but shorter to reduce the heat produced by fermentation in the rumen. These adapted breeds also demonstrate greater resistance to water deprivation than non-adapted ones, tending to drink large amounts of water at once when available.

In terms of reproduction, the reproductive cycles of small ruminants are often regulated by environmental factors such as photoperiod, as well as by nutrition and general physical condition. Undernourishment, sometimes a consequence of a scarce water resource, can cause a delay in follicular growth and changes in oestrous behavior, as well as changes in concentrations of reproductive hormones and ovulation rates. In particular, sheep indigenous to tropical and subtropical regions tend to reproduce throughout the year, but their sexual activity may decrease in summer, when the temperature is higher and the availability of water and food is lower. In arid and semi-arid climates, the breeding season is generally limited to a more favourable time of year, with parts occurring mainly when climatic and food conditions are more suitable for the survival of newborns.

Morphological adaptation:

In the context of morphological changes, we see how the analogies with the adaptation to hot stress seen in the first chapter are very numerous and often linked by a cause-effect phenomenon. Sheep and goats show significant adaptations to manage environmental challenges and water stress, along with thermal stress, is definitely one of the most relevant. Goats, in particular, are adapted to survive in arid and semi-arid environments due to their unique morphology and physiology. These animals, smaller than their European counterparts, have an advantage in terms of heat dissipation due to their relatively larger body surface. This is crucial to reducing water demand in low water environments.

The fleece of small ruminants plays a fundamental role in controlling body temperature. It functions as a thermal barrier that modulates the effects of ambient temperatures, creating a milder microclimate around the animal’s body. This mechanism helps maintain a homeostasis without excessively resorting to evaporative cooling, such as wheezing, which would otherwise cause a significant loss of water.

In addition, fleece and hair color plays a role in solar radiation reflection; lighter colors absorb less heat, helping to keep the underlying skin cooler and reducing the need for heat dissipation through water-consuming processes.

Another significant adaptation is the localization of fat reserves, as observed in sheep in arid regions that tend to store fat in specific areas such as the tail. This feature not only helps in heat dissipation but also serves as an energy reserve during periods of food shortage, further reducing the need for water.

These morphological and physiological adaptations highlight how goats and sheep have evolved and are continuing to evolve to efficiently manage limited water resources (Chedid et al., 2014).

Physiological adaptation:

Ruminant breeds in arid regions have developed specialized physiological mechanisms to conserve water during periods of heat and drought. These adaptations include the ability to produce more concentrated urine, thanks to the extended length of the Henlé loop in their kidneys, which allows to reduce the volume of urine and keep moisture in the stool to a minimum. An example of this is the desert Bighorn which produces a particularly concentrated urine thanks to a thicker than average kidney marrow. Similarly, the Awassi sheep shows a remarkable ability to concentrate urine during periods of poor water availability and can drink large amounts of water when re-hydrated, without disturbing the internal balance of the animal's body.

In addition, during dehydration, there is an increase in urea retention in the kidneys and an increased concentration of urea in the blood. The rumen plays a crucial role as a reservoir, providing most of the water needed during dehydration to maintain blood volume. This process also includes efficient saliva recycling and water and sodium retention in the kidneys, facilitating gradual re-hydration and minimising the loss.

These adaptations show that ruminant breeds in arid regions are optimised to survive under conditions of limited water availability, carefully regulating water intake and storage to maintain homeostasis and survival in hostile environments.

Another aspect concerning the physiology of these animals is about the interdependence between water intake and food consumption. It has been shown that changes in the osmolality of body fluids, caused by the ingestion of food, can significantly influence the behaviour regarding the drinking and eating of these animals. For example, during feeding there is a condition of hyperosmolality and hypovolemia due to the secretion of gastric juices and saliva, prompting animals to drink during the meal or, in conditions of severe dehydration, to reduce food intake.

It has been observed that adequate water intake is essential also for the proper functioning of the digestive system of ruminants, even if water is not strictly necessary for swallowing or humidifying food, thanks to the body's ability to recirculate water from the blood to maintain elevated salivation. However, water remains crucial to compensate for unavoidable losses through excretion and evaporation.

It was also found that water restriction affects the amount of food consumed, similar to what has occurred in situations of thermal stress. For example, Awassi sheep under intermittent irrigation show a significant reduction in food consumption. This reduction is more marked when low quality fodder is offered. In conditions of water scarcity, a slowdown in the movement of food through the digestive tract and an increase in retention time occurs. However, the specific effects of the water restriction on food consumption and digestion need further research to be fully understood (Chedid et al.,2014).

The adaptive characteristics of ruminants, including those just addressed, allow them to exploit pasture areas far from water sources, preventing erosion, especially in regions with water scarcity and high pasture pressure. The decrease in dietary intake under water stress conditions forces adapted ruminants to reduce the metabolic rate to adapt to new body conditions with lower maintenance needs. Dietary restriction for only 3 days causes significant metabolic changes in infant sheep, affecting heat tolerance and, consequently, reproductive potential.

Weight loss is the most obvious physiological consequence of water restriction. However, the adaptability of more rustic sheep to intermittent hydration shows a remarkable resilience of the same; with a water restriction of 3 days up to a month, there was a loss of only about 16.8% of body weight in a month. Weight loss in response to food and water stress is associated with body water loss and fat mobilization, used for energy metabolism in response to reduced food intake. Water restriction causes more weight loss than food restriction alone, although individual variations within the experimental groups show the need for further research to fully understand the impact of this type of stress on weight loss (Chedid et al.,2014).

Table 2: Effect of water restriction on body weight of Awassi sheep.

Physiological status	Water restriction regime	Drop in weight (%)	Age	Ambient temp. (°C)
Non-lactating	2-day-restriction	0.84	mature	15-32
	3-day-restriction	9.98	mature	27-30
		16.8	mature	23-28
		16.7	mature	27-31
		26.2	2 years	30-31
		10.4	mature	18-21
	4-day-restriction	3.32	mature	15-32
	1L on day 4 and 3L on day 8 of 12-day water restriction	22.13	mature	25-35
	1L on day 4 of 7-day water restriction	16.8	mature	23-33
Lactating	3-day-restriction	26.2	mature	27-31

Source: Water Stress in Small Ruminants (Chedid et al.,2014).

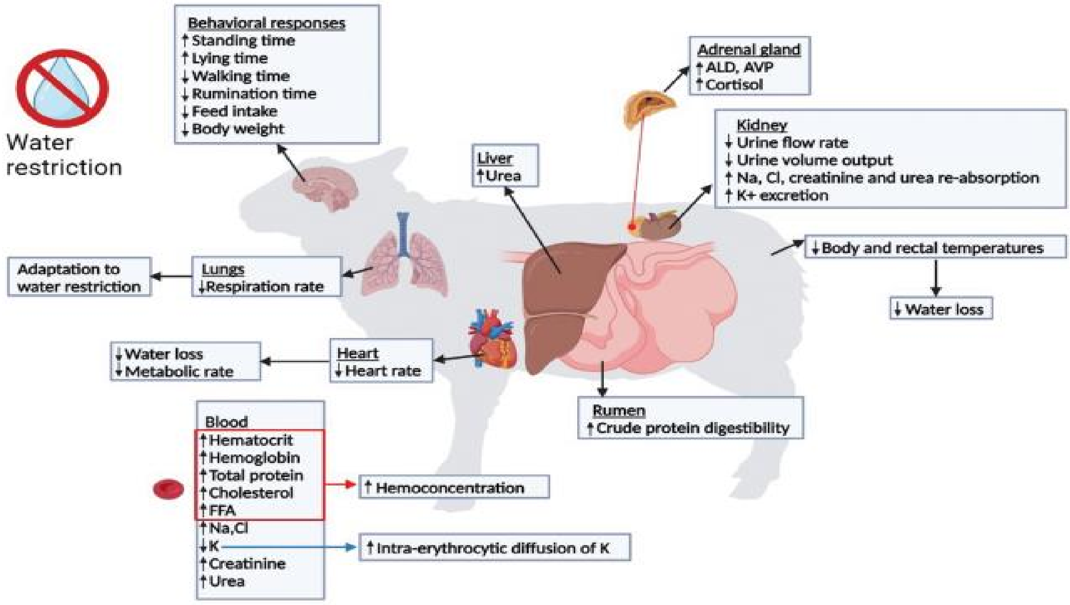
Production capacity:

Water and thermal stress has a significant impact on the productive capacities of animals, particularly in ruminants such as sheep and goats. Reducing food intake under water stress contributes to less water loss but also leads to a decrease in the energy available to animals. This leads to a mobilization of fats, resulting in increased concentrations of cholesterol, cortisol and free fatty acids in the blood, as observed in studies on sheep and goats.

On the reproductive front, water stress can cause a number of problems. In male specimens of the Barbarine breed, for example, a decrease in libido and testosterone concentrations was noted, while in females a delay in follicular growth and a reduction in the response and duration of oestrus was observed. However, some breeds such as Awassi sheep show remarkable adaptability, with the ability to maintain pregnancy even in conditions of water shortage. With regard to meat production, severe water shortages can reduce body weight at slaughter and thus adversely affect the physico-chemical qualities of meat, such as color, juiciness and tenderness. However, moderate water restrictions (up to 33%-40% of voluntary intake) appear to be tolerated by some breeds, without significant negative impacts on production performance.

In terms of milk production, water stress generally reduces yields on milk due to a slower breast blood flow, slowed by water shortage. This reduction is often accompanied by an increase in the concentration of lactose, fats and mineral salts in milk. However, the resilience varies depending on the breed: Aardi goats, for example, show a remarkable ability to adapt to water restrictions up to 50%, while Lacaune dairy sheep are less tolerant, with a capacity to withstand water restrictions only up to 20%.

Fig.2 Physiological changes in sheep under water restriction



Source: Mitigation and animal response to water stress in small ruminants (Pérez et al.,2023).

In conclusion, water and thermal stress have a considerable influence on the productive performance of the animals, with effects varying according to species and breed. Reduced food intake, changes in lipid metabolism, changes in reproduction and growth, as well as the impacts on the quality of meat and milk are all critical aspects that must be considered in the management of livestock in stressful environments (Pérez et al.,2023).

2.2 Water restriction and consequences on livestock systems

The global situation regarding water resources is extremely critical and complex. Only a small fraction of the water on Earth is fresh and available: about 2.50% is fresh water, but much of it is trapped in glaciers and ice caps. Only 0.77% is accessible in aquifers, lakes, rivers and other surface water bodies. Although water is fundamental to human life, its availability is decreasing drastically. The pressure on water supply is aggravated by a number of factors: increased world population, industrialisation, climate change and increased use in areas such as agriculture and livestock.

Of all phenomena related to climate change, water scarcity poses a significant challenge in many respects, involving more than 40% of the world's population and more than 1.7 billion people living in water-restricted areas. This problem is set to grow globally as a result of ever-increasing climate change, demographic growth, water pollution, changes in land use and economic dynamics (Akinmoladun et al.,2019).

The most vulnerable regions are arid and semi-arid regions, where the economy is heavily dependent on natural resources and climate-related activities. In these areas, rainfall is expected to decrease by 20% or more in the next century. The reduction of water resources will lead to a decrease in agricultural production and an increase in prices, strongly influencing food security and economic development. In addition, this will force farmers in particularly vulnerable areas to make a choice as to the type of livestock best suited to the expected scenario.

To date, a quarter of the global economic impact caused by climate extremes has been absorbed by the primary sector, where the livestock system, alongside the crop subsector, suffers most of the total damage and losses. The livestock sector has recently had the lowest annual increase in meat production (1 percent in 2016). The future of the sector, in fact, will be increasingly challenging with the expected scarcity of crucial resources for production, in particular land and water. Climate change leads to reductions in livestock productivity by directly depressing animal adaptive response mechanisms, altering the spread and burden of disease, and causing heat stress and related welfare issues; and indirectly affecting the availability of forage crops and the quality of forage. Therefore, generating research evidence on the impacts of climate change and adaptation on livestock will have important implications for the development of the sector and the people who depend on it (Escarcha et al.,2018).

The Fourth Assessment Report of the Intergovernmental Panel on Climate Change stresses that stress on water resources will increase without adequate mitigation strategies. Particularly vulnerable areas with limited water resources are found in North Africa, Northeast China, Pakistan, India, North America and the Middle East (Akinmoladun et al., 2019).

Currently, the difficulty in meeting the demand for good quality water has reached critical levels in many parts of the world. This raises significant concerns about meeting water requirements for food production for the growing world population, estimated to reach 9.8 billion by 2050.

Livestock, in particular, is severely affected by the unavailability of suitable drinking water, becoming a priority concern for farmers. Low rainfall affects not only the availability of water for livestock, but also reduces access to food sources such as meadows and pasture for small ruminants, especially in semi-arid areas.

The choice of animal species to breed becomes crucial in these changing conditions. Small ruminants emerge as a promising solution due to their low production costs, shorter reproductive cycles, adaptability to small soils and the ability to efficiently use crop residues. Their tolerance to adverse climatic variables, such as low rainfall and thermal stress, makes them preferable to livestock and other animal species.

The correlation between drought and preference for small ruminants is evident in pastoralist practices in different regions, such as Kenya and Ethiopia, where the increasing frequency of droughts has led to increasing dependence on these animals, as they demonstrate greater resistance to adverse conditions than livestock and require, as mentioned above, a smaller amount of water.

As regards the impact on productivity, when lactating sheep and goats are deprived of water for 72 hours, there is a variation in milk production in 50% of sheep, showing an increase in the viscosity of milk and its components such as lactose, fats, proteins and mineral salts. In another study, Comisana ewes subject to water restrictions for 40 days with varying percentages of water intake did not show a quantitative reduction of milk, but research attributes this to the low water demand caused by the low environmental temperature. The black Bedouin goats, instead, adapted to the desert, demonstrate a remarkable resistance to the scarcity of water, maintaining a milk production of 70% compared to the norm even after four days of restriction followed by two days of rehydration.

The tendency to decrease milk production under water restriction conditions is associated with several factors, including a reduction in blood flow and a decrease in prolactin secretion. However, the black goat Bedouina autochthonous of the desert, shows an efficient use of the water even with higher restrictions (50% compared to 25%), highlighting the importance of the same in influencing the production even in arid contexts.

The water restriction also impacts on food intake, with a 10% decrease in food consumption in Moroccan goats in two days of water deprivation. However, some more resilient breeds keep their food intake unchanged even during limitation, suggesting an adaptive mechanism to ensure adequate milk supply during periods of water shortage.

Adaptation to a prolonged decrease in water also involves vasopressin, a water saving mechanism, resulting in some changes in milk components such as increased urea concentration, sodium and chloride in proportion to the intensity of water deprivation itself. Reduced milk production during this phase is also associated with changes in the blood metabolites of lactating sheep, such as a decrease in the concentration of hemoglobin and hematocrit pressure, suggesting an adaptive response to maintain homeostasis during dehydration. In summary, water stress also has significant impacts on milk production, which is one of the most important aspects within an intensive or extensive farming system, affecting the composition and quantity of the product, and the consequent adaptive responses vary according to races and environmental conditions (Akinmoladun et al.,2019).

2.3 Strategies to mitigate water and heat stress

Specific nutritional strategies are adopted to mitigate the impacts of thermal and water stress on the feeding behaviour of ruminants. Water restriction, for example, causes reduced food intake due to the close correlation between water intake and dry matter, therefore, it is essential to provide a high quality diet to ensure optimal nutrient intake. Studies have shown that 50% water-restricted Mutton Merino rams had a similar intake of food compared to unrestricted conspecifics, but those fed with medium protein reported slightly higher gains.

In addition, the effects of some supplements have been evaluated as factors mitigating compared to water stress. Although vitamin C, for example, has been studied for its antioxidant capacity and its administration has shown positive results in several species, use in ruminants is not common due to their ability to synthesize it normally. However, studies such as those conducted on Awassi sheep show that the administration of vitamin C under water stress can reduce weight loss and improve some blood parameters.

In addition to vitamin C, the integration of antioxidants such as selenium and vitamin E has been studied to limit the effects of thermal stress. This strategy has been shown to reduce adverse effects through the suppression of inflammatory genes and an improvement in oxidative state.

On the other hand, the addition of fats in the diet, with a reduction in starch and fiber, has been identified as an effective strategy to decrease the metabolic heat and rumen during thermal stress. Polyunsaturated fatty acids (PUFAs) included in the diet have been associated with an improvement in immune function during conditions of this type of stress, as indicated by several studies in sheep.

In conclusion, although vitamin C and E can be a useful mitigating factor against water stress, it is essential to continue research on supplements and additives of different nature that can be more practical in the application, especially in extensive management systems without easy access to water. Overall, the exploration of targeted nutritional strategies is a growing field to mitigate the impacts of thermal and water stress on small ruminants (Pérez et al.,2023).

3. OBJECTIVES

3.1 Introduction at the trial

In a global context where climate change is radically changing environmental balances, it is crucial to understand and anticipate the impact that such changes can have on the livestock sector. The Mediterranean sheep, for example, is at the heart of an industry that is based on extensive or semi-extensive management systems, with milk and meat production often transformed into typical and local products. Such systems, especially those located in the southern Mediterranean basin, depend on atmospheric phenomena such as abundant rainfall that allow the growth of lush pastures. Among the most critical aspects, in fact, is the growing uncertainty about the availability of water, a factor that can have significant impacts on the welfare and productivity of the animals reared. In this scenario, this thesis research aims to test the resilience of a sample of 40 sheep of Aragonesa breed, which was found to be particularly resilient in the presence of hostile climates thanks to a series of behavioral and physiological adaptive mechanisms (Lacosta et al.,2020). To do this, the animals were categorised into two different groups of 20 sheep each and treated according to a condition of high or low stress tolerance according to assessments made before the start of the test.

The core of the study focuses on the analysis of how two different diets, one of which is associated with a voluntary administration of parasitic larvae (intended to the twenty high-strength samples) and both formulated according to the degree of stress resistance mentioned above, can affect the welfare of sheep under water restriction conditions.

Parasitic infection is an important element in the experimental procedure because it reflects a real problem; grazing sheep are increasingly exposed to a wide range of pathogens, in particular gastrointestinal nematodes (GIN), with significant economic impacts. To this is added the fact that the parasites have developed an increasing resistance to anthelmintic and that therefore an alternative solution had to be used (Lacosta et al., 2020). As a result of this need, it has emerged from some studies that sainfoin (Onobrychis viciifolia), a Mediterranean legume, is particularly prone to this purpose for its anthelmintic properties. The Aragonesa sheep trial showed promising results, with delays in the onset of helminths and coccidia infections. In addition to this, sainfoin offers additional benefits, such as the high content of polyunsaturated fatty acids and antioxidants. These components are relevant to strengthen the immune system and address the effects of stress (Lacosta et al.,2020).

In parallel, to monitor the impact of parasitosis and water restriction on subjects tested, blood samples and regular body weight and body condition score (BCS) assessments were carried out, with the aim of obtaining a detailed picture of physiological responses and behavioural changes induced by water restriction, including changes in food intake.

Through this quest, aims to provide valuable insights into the resilience of sheep in environmental stress scenarios and to contribute to the development of more effective management and adaptation strategies for breeding these animals in a future of increasingly pressing climate and environmental challenges.

3.2 Aims of the trial

The purpose of this thesis is to investigate the effects of water restriction on sheep belonging to the Aragonese breed, subjecting them to two different diets. The experiment, which lasts a total of about 11 weeks, includes the period of adaptation of the animals in the boxes before the administration of the larvae, until its conclusion with the sacrifice of the samples.

The experimental approach is to divide 40 sheep into four distinct groups, each of which has different combinations of factors: high stress resistance with straw and concentrate administration, low resistance with straw administration and concentrate, high stress resistance with sainfoin administration and low stress resistance with sainfoin administration. The sainfoin was administered to 20 animals previously chosen for parasitic infection, with the aim that this plant mitigates its effects on the body.

During the 11 weeks of testing, weekly samples were taken for Body Condition Score (BCS), live weight, water and forage ingestion for all 40 samples. This systematic approach made it possible to monitor the evolution of these parameters over time, providing a detailed overview of the physiological and behavioural responses of animals to stressful factors.

The main objective is to determine a slope and an overall trend of these values by comparing the four groups with each other, using statistical and graphic tools to make clear the correlations between the groups themselves. The major focus is on understanding any significant differences between the two main macrogroups, namely animals fed with concentrate and those with lupin. This comparative analysis concerns only the in vivo parameters mentioned above.

4.MATERIALS AND METHODS

4.1 Materials

Animals:

Forty Aragonese breed sheep aged from 2 to 8 years old from the Soto Lezcano flock were used for this study, the spread of this breed is concentrated in the Ebro Valley, mainly in the autonomous community of Aragon and in regions of other communities, such as Castilla y León, Castilla-La Mancha, Navarra, La Rioja or Catalonia. The study was carried out at the Agri-Food Investigation Centre of the Aragon region (CITA) in the city of Zaragoza. The sheep involved in the experiment were selected based on results from a test previously conducted on 200 sheep in May 2022, which involved subjecting them to a 5-day water restriction. All blood and wool results collected from the animals during the preparatory phase were studied. The ratio of neutrophils to lymphocytes in the blood and the trend of cortisol in the plasma, adjusted for live weight and body condition, were used with the aim of creating a group from which to choose the sheep more or less tolerant to stress. Similarly, the degree of kinship in the batch that proved suitable was taken into account, as it was less than 10%.

Fig. 3: Sheep flock of Rasa Aragonesa breed in "Los Monegros" a dry region of Aràgon, Spain.

Source: Mitigation and animal response to water stress in small ruminants (Pérez et al.,2023).

The experiment evaluates two effects: the animal's level of stress tolerance and the integration of sainfoin in the diet, to ascertain whether or which parameters can modulate the imposed water stress and parasitism. The animals were divided into four different groups:

High tolerance + control (n=10)	High tolerance + sainfoin (n=10)
Low tolerance + control (n=10)	Low tolerance + sainfoin (n=10)

Diets:

Before the start of the experiment, all sheep will receive a basal diet with a 70:30 ratio of straw to concentrate (rum Oviaragaon). The straw and concentrate diet, as it would have resulted in a non-homogeneous mixture, was distributed by separating the two components. Once the test begins (on day 0), half of the sheep will receive sainfoin pellet instead of the control concentrate.

- The control group will eat: 1 kg of straw and 300 g of basal concentrate.

- The sainfoin group will eat: 1 kg of straw and 300 g of sainfoin pellet.

Both the straw and the concentrate were provided to the sheep in designated areas within the cage, arranged in such a way that the two components could not mix. The excess straw was collected in boxes placed under the feeder, each numbered according to the corresponding animal. Additionally, the daily straw waste was weighed and recorded, again for the purpose of evaluating the intake pattern for each individual sample.

Moreover, all sheep will have access to mineral blocks ad libitum.

Water:

The water restriction begins on the seventh day following the administration of parasitic larvae. Until that moment, water availability was "ad libitum," and it was provided inside drinking troughs arranged in the adaptation boxes. These boxes were arranged in four rows, so the animals at the beginning and end of each row had a single drinking trough, as they were at the "vertices," while the animals in the middle shared the administered water, accessing it from the same trough positioned in the centre of the two boxes.

Water intake was monitored using counters installed on each cage. The device calculated the difference between the water offered and the residual liquid. Concurrent with the start of the trial, some counters malfunctioned, so the water counting became manual: 1.25L was administered to animals in single boxes and 2.5L in double boxes. The residual liquid was measured using syringes and graduated beakers. Following the same principle as the counters, the water intake was calculated.

4.2 Methods

Management before the test:

- The sheep will be dewormed 30 days before the start of the experiment through anthelmintic treatment by administration of ivermectin (0,2 mg per kg LW) and albenzadol (20 mg per kg LW).

- 10 days before the test begins, the sheep will be placed in the boxes with a grid floor where they will stay for the duration of the test, to begin their adaptation period. The sheep will be weighed beforehand and their body condition will be recorded in a dedicated diary. During this adjustment period, all will be fed the same diet.

- The test will start on day zero, at which time half of the animals will be infected with L3 Haemonchus larvae and the same subset will be given sainfoin pellet instead of the control concentrate. On the 7th day, a 40% daily water restriction will begin compared to the water consumption in the previous week, which will last for 28th day, until the 35th day of the trial. The trial will conclude on the 56th day with the commencement of the sample culling.

Daily management:

As previously mentioned, the start of the water restriction coincides with the seventh day after the administration of Haemonchus L3 larvae, and in this specific experiment, this date falls on 9th May 2023. From this moment, the standard daily procedure includes:

- Arrival at 8:30 AM at the barn where the animals are housed, using graduated syringes to collect and calculate the remaining water in the drinking troughs. The water intake is then recorded in the appropriate diary, determined by the difference between the water supplied the previous day and any remaining the following morning, if present.

- Once the difference is calculated and noted, the troughs are manually cleaned of any straw and/or faecal residues and refilled with the chosen dosage (previously mentioned). During the 28 days of water restriction, water is supplied once a day according to this schedule.

- Subsequently, still in the early morning hours, the leftover straw deposited in the boxes under the feeders, which are numbered per sheep, is collected. With the help of a scale, the amount of forage consumed by the animal during a day is calculated. The quantity in grams of the “leftover” is then noted in the appropriate diary, in order to study the feeding behaviour of the sheep in relation to other parameters (H2O intake, THI, temperature).

- Regarding the practical part, day by day, bags containing straw and concentrate are distributed to the samples and replenished so as to have enough for as many days as possible.

- Cleaning operations of the barn are carried out using water pumps which, thanks to the jet's power, direct feces and other dirt into the designated disposal channel.

Weekly management:

- Starting from day 0, the experimental day coinciding with the administration of parasitic larvae, measurements are also taken on the live weight of the animal and its body condition score (BCS). The animals are individually brought onto a platform equipped with a scale that immediately displays the sample's weight. Once the access site to the platform is closed, a manual palpation is performed on the lumbar region of the animal to determine the BCS score, which can range from 1 to 5 depending on the recognized adipose deposition.

- In addition to the aforementioned parameters, whose measurements are consistently repeated throughout the weeks of the test, other indices alternate among them; blood, feces, saliva, and wool samples are collected to understand the animals’ level of stress.

- Blood samples were collected from each individual animal through its immobilization, and after applying pressure to the carotid area to make the vein more visible and accessible to the needle.

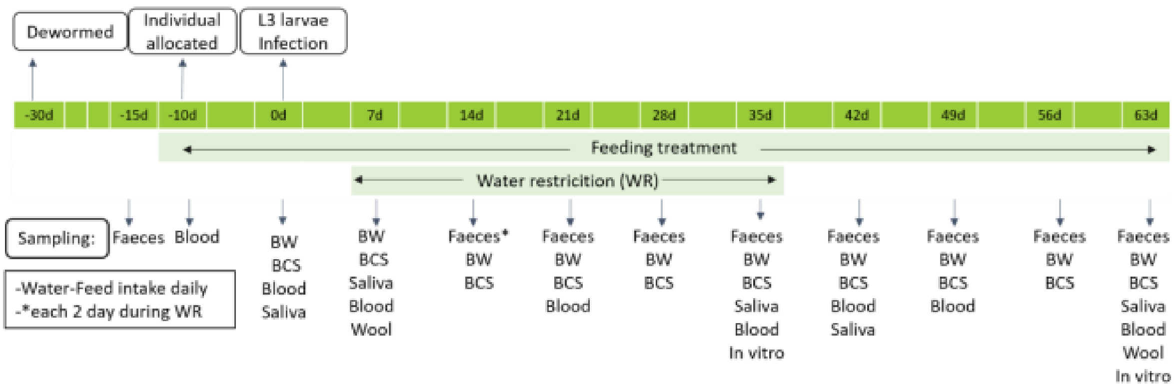
- The faeces were similarly collected by immobilising the animal and, with the help of a plastic bag numbered according to the sample, were taken directly from the rectum.

- Saliva sampling is carried out using swabs rubbed inside the cheek, which are then drained into 15 ml falcon tubes and taken to the laboratory. The goal is to determine the concentration of parasite-specific IgA. Saliva is recovered by centrifugation and frozen before ELISA analyses.

- Wool samples are taken from the left scapular region, at the beginning and end of the test, using a razor.

The latter procedures have been described as part of the experimental protocol, but will not be relevant for the analysis of this research thesis.

Fig. 4: Experimental protocol on timeline (Lacosta et al., 2020).



- Blood analysis includes:

Tube with EDTA (lavender) 5 ml for:

- Complete Blood Count (CBC)
- Total protein count

Tube with heparin (green) 10 ml for:

- Cortisol, DHEA, DHEA-s
- Glucose (to be shown to the veterinarian)
- Fatty acid count (with the addition of 2 ml of plasma)
- Carotenoids and cholesterol (with the addition of 1 ml of plasma)
- Antioxidants

Tube without anticoagulant (red):

• IgA count

Environment and animal welfare:

- With the arrival of the animals for the adaptation period, two sensors were placed inside the shed in order to record the temperature and humidity level throughout the test period. The temperature was 22 C° with a humidity level of 56%. With the reported data the THI will be calculated. The levels of cortisol in the blood, an important factor in determining the stress condition, will therefore also be evaluated according to the climatic condition in that particular day or period.

- Overall the farming conditions were quite good. The lighting was of a natural type, the animals were in boxes wide enough to be able to stand or decubitus with a grid structure so that they could see their own kind. At the expense of this, the ventilation was also natural and consequently the sheep were not always comfortable with the temperature in the shed. The latter was always kept open during the day to allow maximum airflow.

- All the experimental procedures performed in this trial were approved by the Animal Ethics Committee of the CITA Research Centre (CEEA, 2021-65), in compliance with the guidelines of Directive 2010/63/EU of the European Parliament and of the Council of 22 September on the protection of animals used for experimental purposes.

Statistical analysis:

- The statistical analysis on such data was conducted through the use of advanced software and regression techniques. To manage the time autocorrelation due to the weekly cadence and the consecutivity of the samples, a correction in the regressions has been implemented. This correction, based on an AutoRegressive Moving Average (ARMA) model, was essential to consider the dependency between successive and previous samples. Statistical analysis has adopted a non-linear approach, made possible by the use of a Generalized Additive Model (GAM). This model allows to describe the trends not only through linear functions, but also polynomials, providing a more complete and detailed representation of the dynamics observed during the experiment.

- The second part of the statistical analysis was conducted using R Studio, enabling the graphical and numerical exploration of the correlation levels among dependent variables within and between groups. Subsequently, an Excel sheet was created to document the significance of the correlations, highlighting positive correlations in green and negative correlations in red.

5.RESULTS AND DISCUSSION

5.1 Results

In this chapter, we will explore in detail the results obtained through the analysis of the four groups defined in the course of our research. To visually understand the evolution of fundamental parameters in vivo, we have created four distinct graphs corresponding to the groups considered. These graphs were compared for four key parameters: Body Condition Score (BCS), Live Weight, Water Ingestion and Food Ingestion. The main objective of this analysis is to observe and interpret graphically how each group has varied over the weeks in relation to each parameter. This approach will allow us to identify trends, significant differences and possible correlations between the variables considered, thus providing a comprehensive and clear picture of the progress of the experiment as a whole.

The legend

Baja-EstrésCONTROL **b_e_c :** group of sheep with Low tolerance + control diet (n=10)

Baja-EstrésESP **b_e_e :** group of sheep with Low tolerance + sainfoin diet (n=10)

Alta-NoestrésESP **a_n_e :** group of sheep with High tolerance + sainfoin diet (n=10)

Alta-NoestrésCONTROL **a_n_c :** group of sheep with High tolerance + control diet (n=10)

Trend of groups over the weeks

Fig. 5: Evolution of water ingestion during weeks in the 4 groups (GAM analysis)

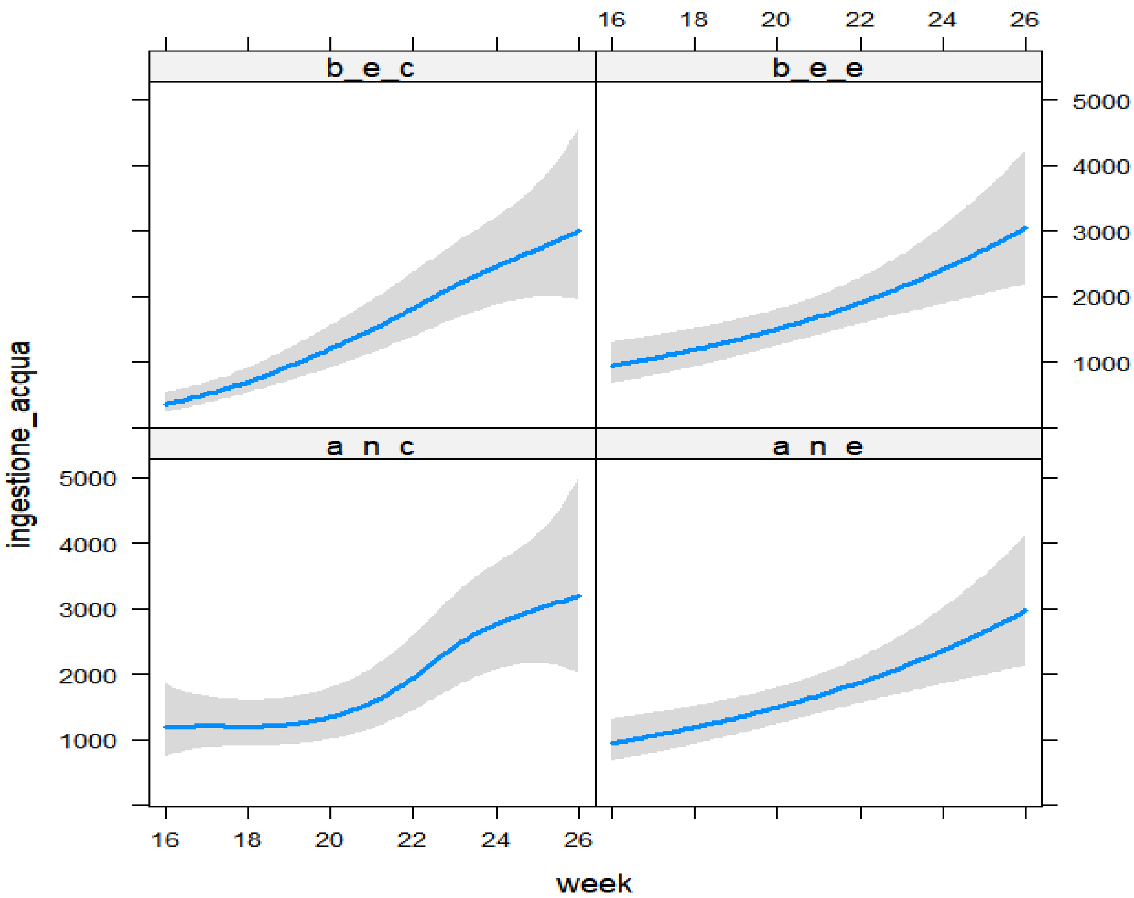


Fig. 6: Evolution of dry matter ingestion during weeks in the 4 groups (GAM analysis)

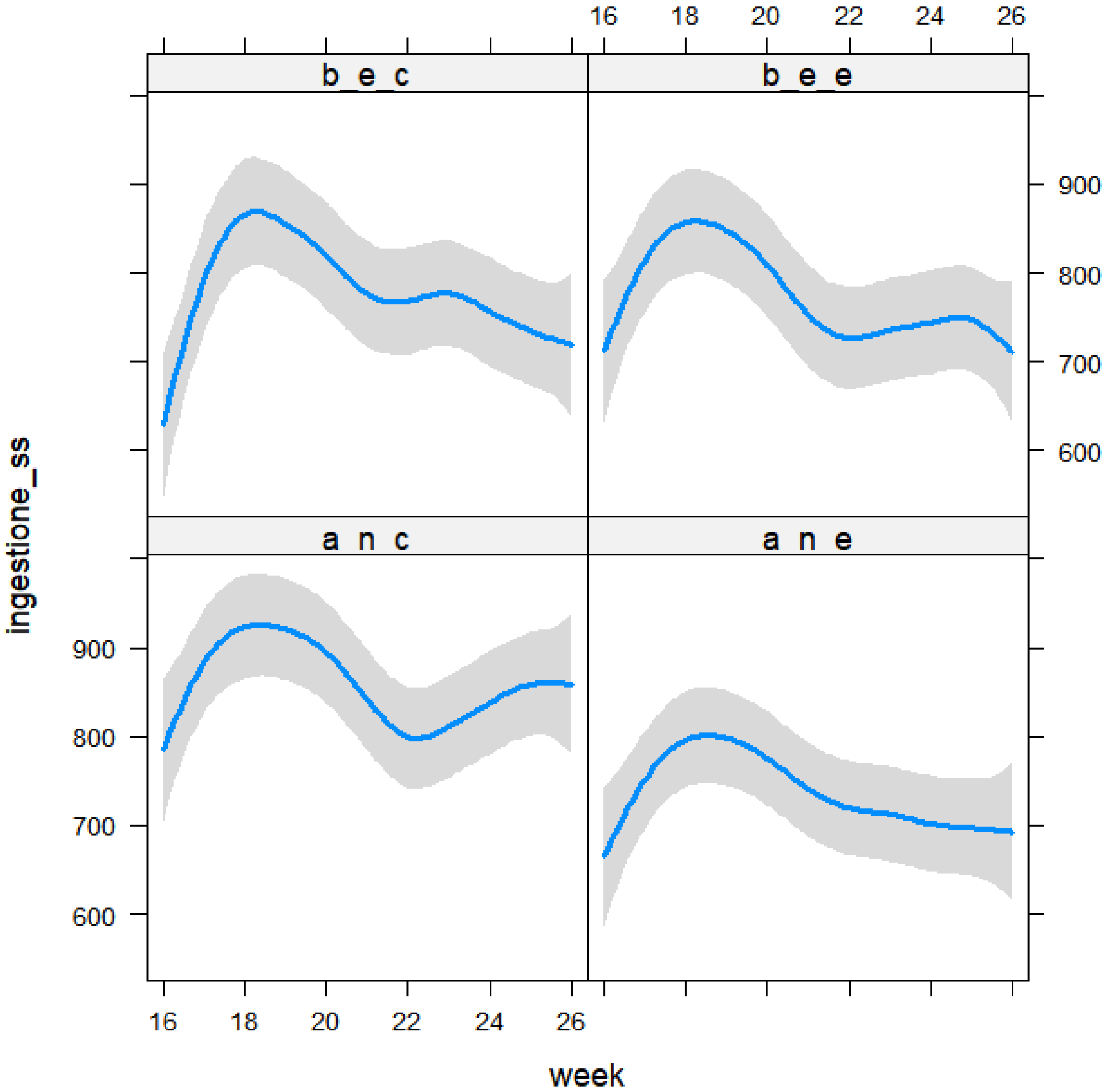


Fig. 7: Evolution of body weight during weeks in the 4 groups (GAM analysis)

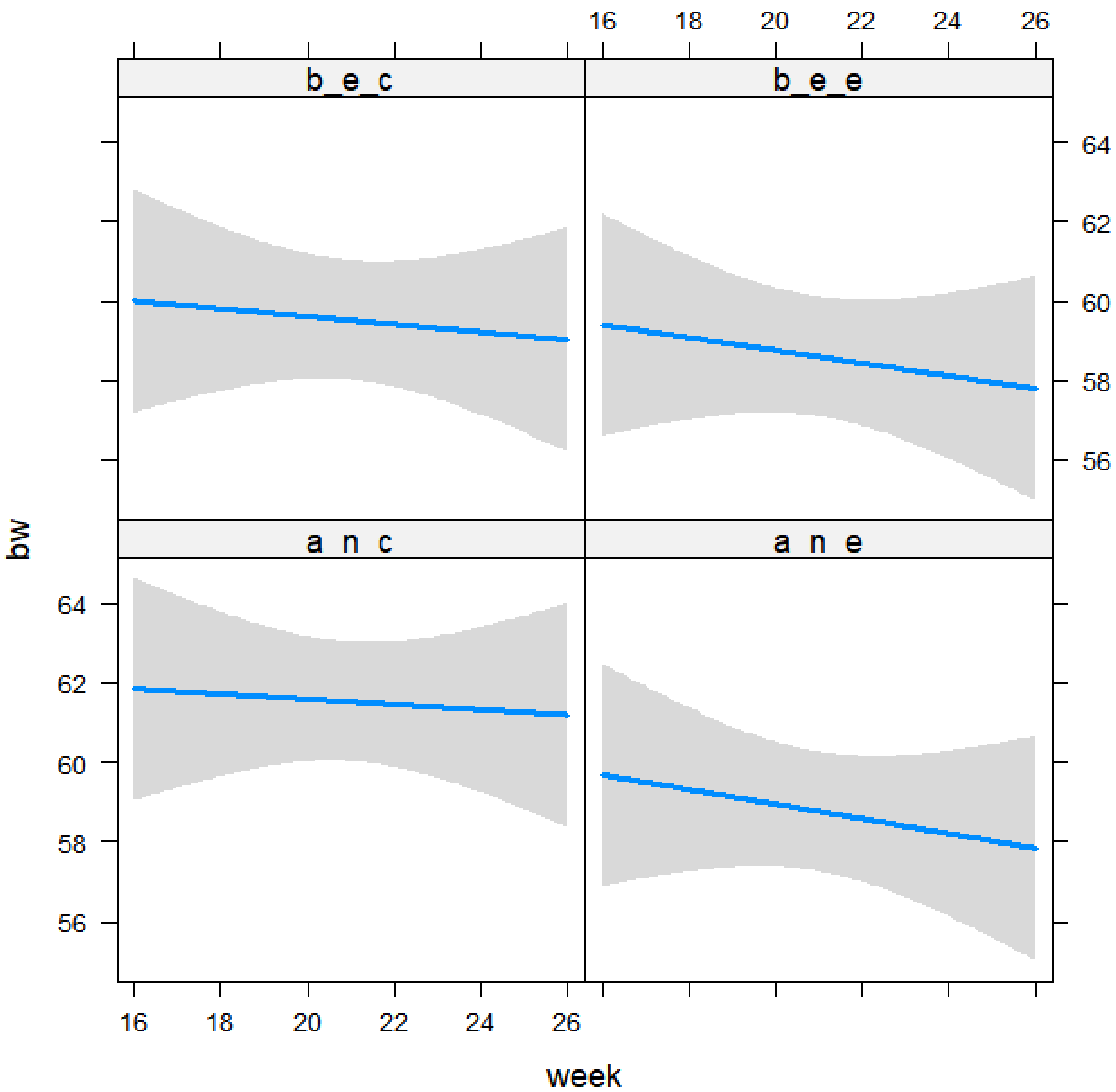
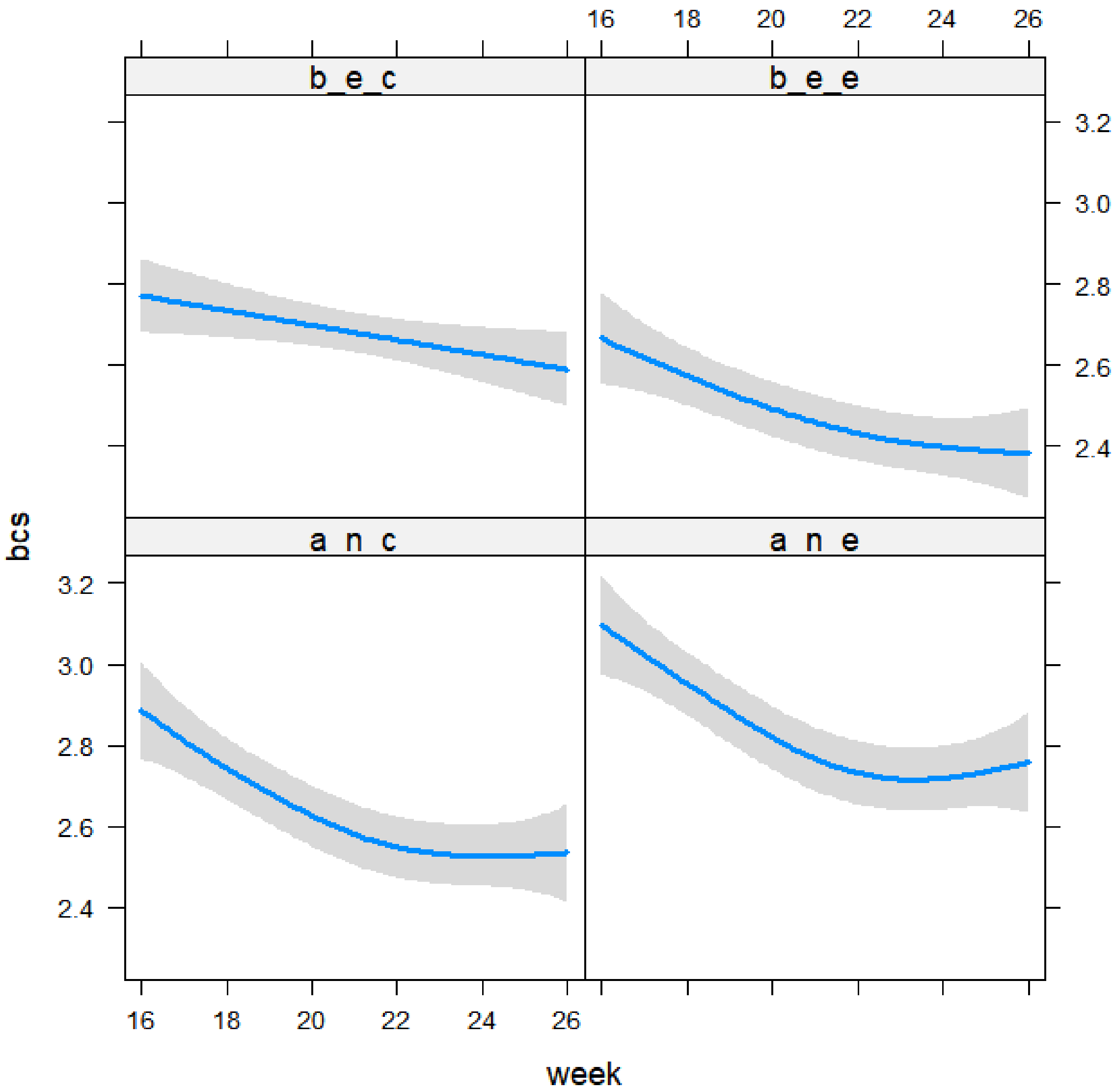


Fig. 8: Evolution of body condition score during weeks in the 4 groups (GAM analysis)



The analysis of these graphs shows a significant difference between the groups for all parameters except live weight, which shows a uniform trend in all four cases. Thus, such uniformity occurs regardless of the different characteristics that define the groups, such as variations in diet and different resistance to stress. Therefore, in this specific context, the live weight does not reveal significant disparities that can be highlighted in the statistical analysis. As a corollary of this statement, we present below an explanatory scheme, providing a numerical representation of the data shown in the graphs, considering ***** as the degree of significance:

Table 3: Effect of group on BCS

	edf	Ref.df	Chi.sq	p-value	
s(week): group **Alta-NoEstrèsCONTROL**	2,204	2,745	9,005	2,78E-05	***
s(week): group **Alta-NoEstrèsESP**	2,4	2,988	9,155	7,51E-06	***
s(week): group **Baja-EstrèsCONTROL**	1	1	5,314	0,02163	*
s(week): group **Baja-EstrèsESP**	1,743	2,172	6,564	0,00119	**
	df	F	p-value		
Group	3	33,3	<2e-1		

Table 4: Effect of group on BW

	edf	Ref.df	Chi.sq	p-value	
s(week): group **Alta-NoEstrèsCONTROL**	1	1	0,074	0,786	NS
s(week): group **Alta-NoEstrèsESP**	1	1	0,058	0,447	NS
s(week): group **Baja-EstrèsCONTROL**	1	1	0,162	0,688	NS
s(week): group **Baja-EstrèsESP**	1,001	1,002	0,437	0,509	

	df	F	p-value
Group	3	3,058	0,0281

Table 5: Effect of group on dry matter ingestion

	edf	Ref.df	Chi.sq	p-value	
s(week): group **Alta-NoEstrèsCONTROL**	4,364	5,398	2,329	0,03543	*
s(week):group **Alta-NoEstrèsESP**	3,753	4,658	2,6	0,034667	*
s(week): group **Baja-EstrèsCONTROL**	4,805	5,925	4,014	0,00713	***
s(week): group **Baja-EstrèsESP**	4,431	5,479	2,952	0,010118	*

	df	F	p-value
Group	3	14,33	6,63E-09

Table 6: Effect of group on water ingestion

	edf	Ref.df	Chi.sq	p-value	
s(week):group **Alta-NoEstrèsCONTROL**	2,546	3,17	19,28	0,00039	***
s(week):group **Alta-NoEStrèsESP**	1,007	1,014	16,15	6,38E-05	***
s(week):group **Baja-EstrèsCONTROL**	2,102	2,62	58,69	2,00E-16	***
s(week):group **Baja-EstrèsESP**	1,002	1,003	17,17	3,55E-05	***
	df	F	p-value		
Group	3	7,896	0,05		

As observed in the numerical representation, the significance of the "liveweight" parameter appears negligible. In fact, the smaller the number corresponding to the p-value, the higher the significance, which is why lower p-values are associated with a greater number of asterisks. Typically, one asterisk (*) is indicated for a p-value < 0.05, two (**) for p-values < 0.01, and three (***) for p-values < 0.001.

It was then decided to correlate the four in vivo parameters within each research group and among the groups themselves to understand if and to what extent they influence each other. Representative graphs were initially created to visually observe these correlations, followed by numerical values presented in a table. The table highlights these values with dark green indicating highly positive correlations and intense red for strongly negative correlations. It is important to note that these coefficients can range from -1 to +1, depending on the strength of the correlation. Moreover, the closer a coefficient is to 0, the more negligible the correlation between variables.

Correlation analysis

Fig. 9: Correlation into ***Alta-NoEstrèsCONTROL*** group*:*

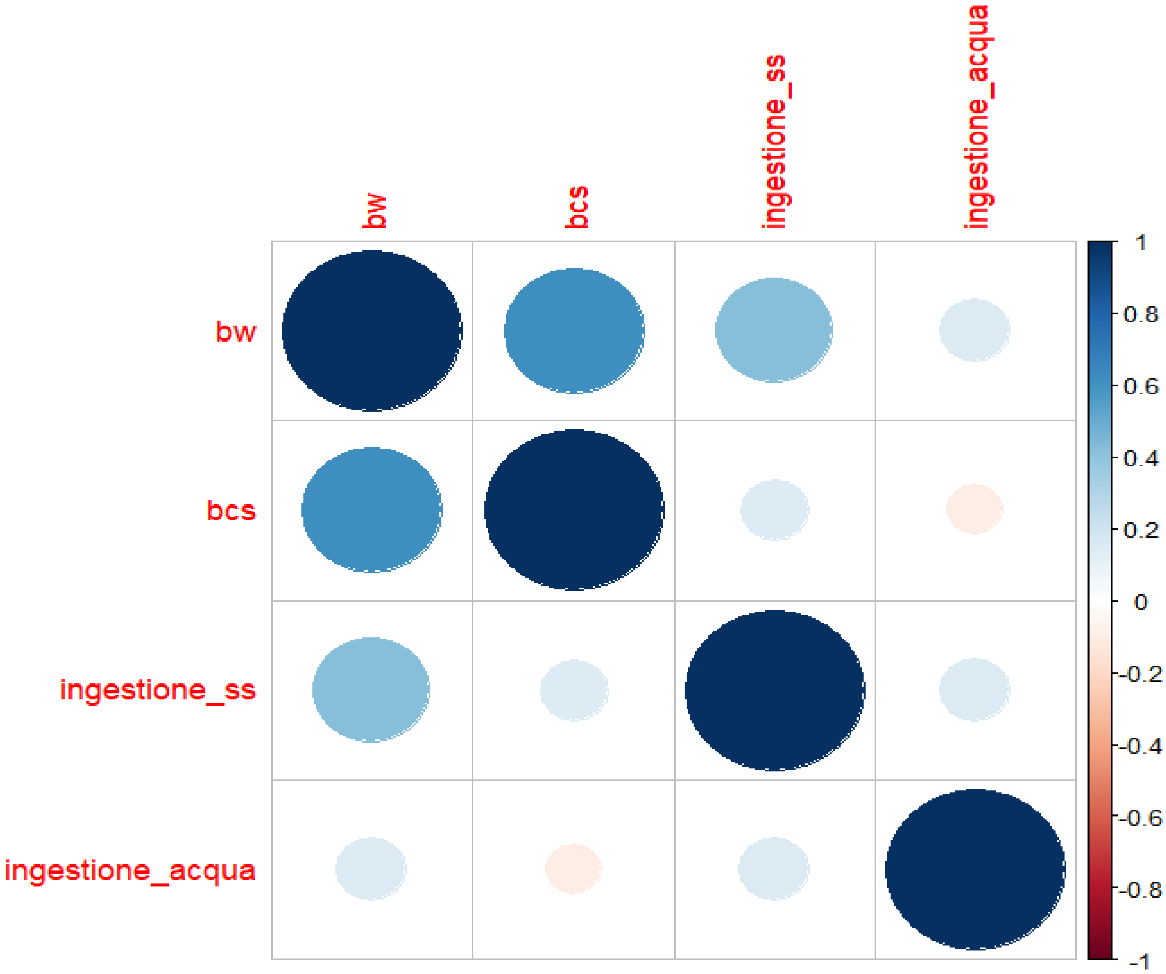


Fig. 10: Correlation into ***Alta-NoEstrèsESP*** group:

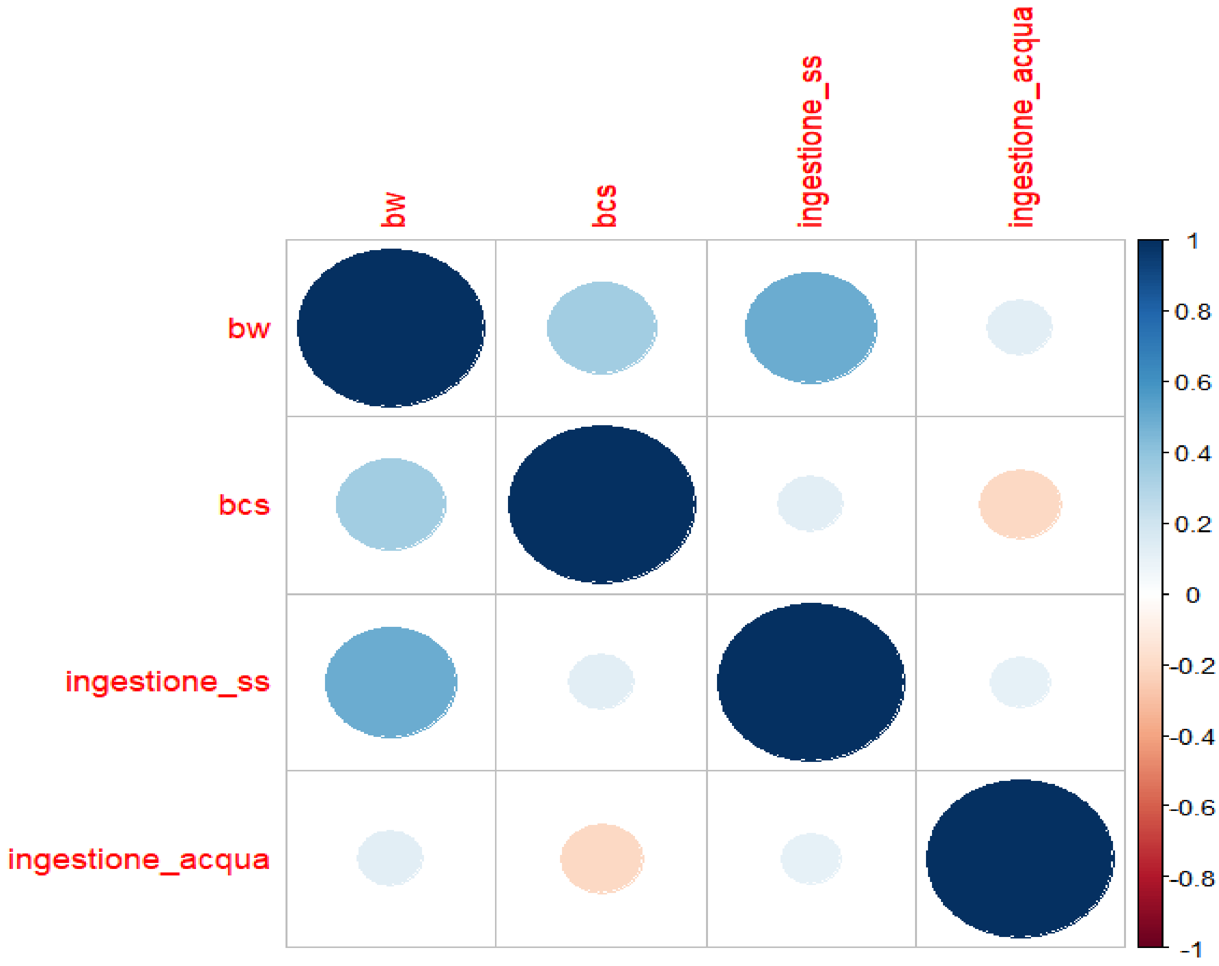


Fig.11: Correlation into ***Baja-EstrèsESP*** group:

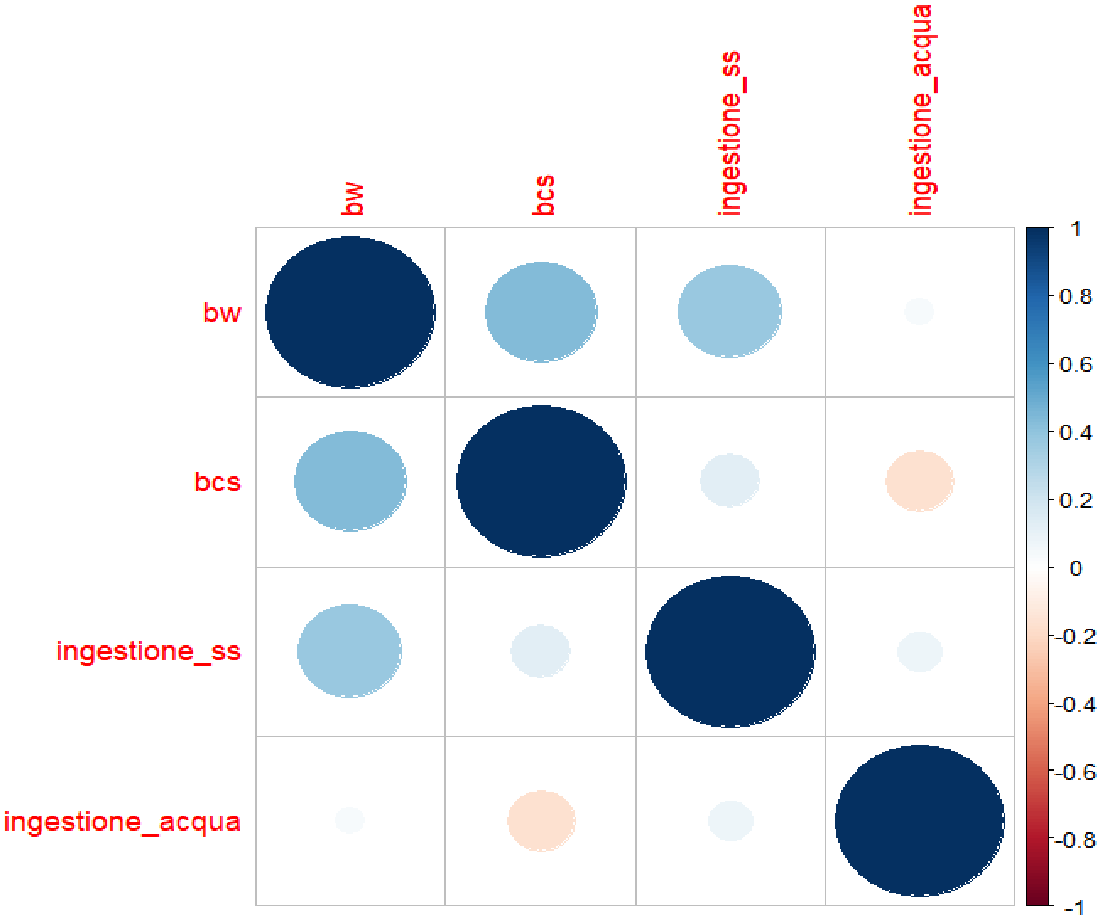


Fig.12: Correlation into ***Baja-EstrèsCONTROL*** group:

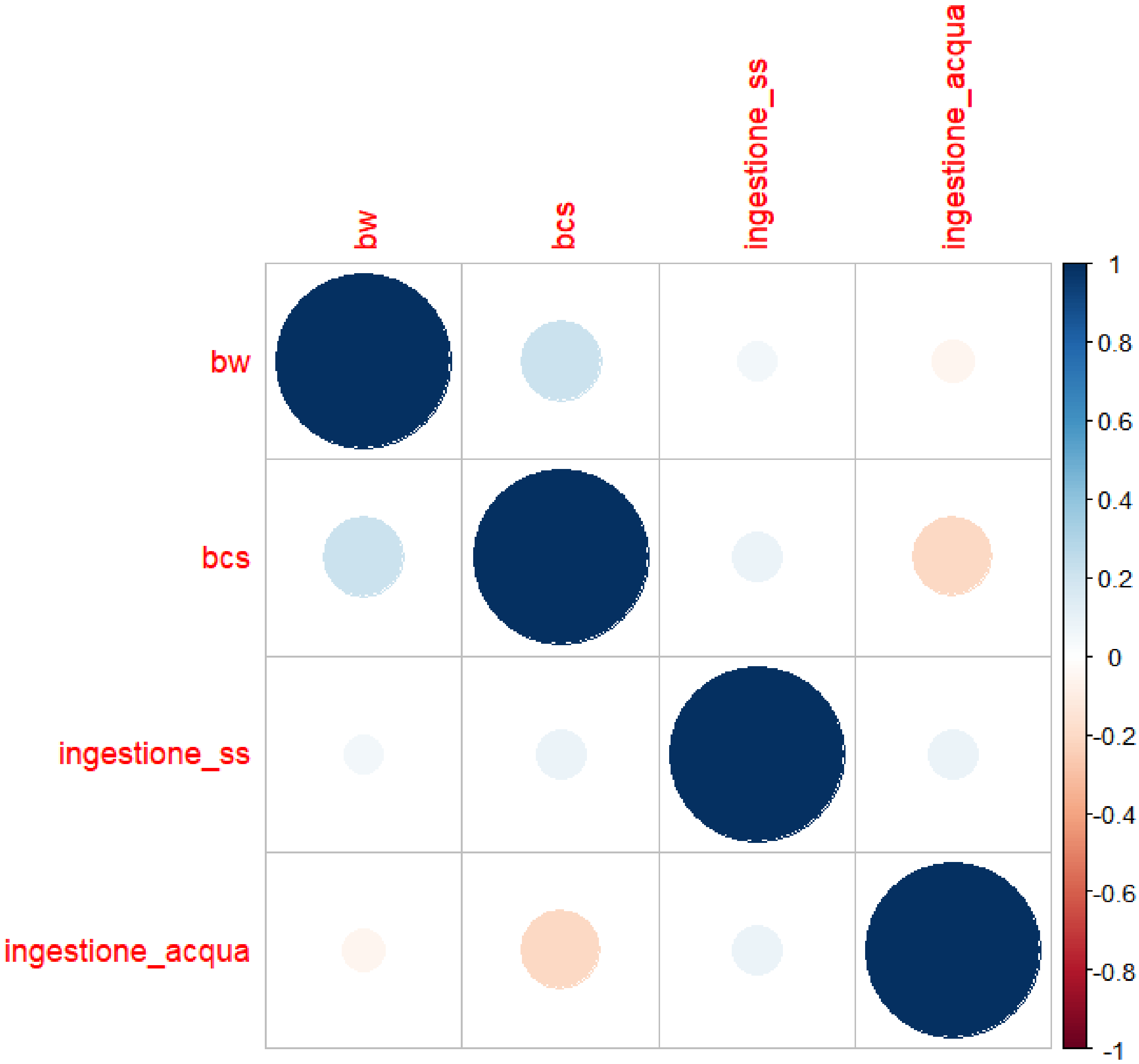

bw
bcs
ingestione_ss
ingestione_acqua
bw
bcs
ingestione_ss
ingestione_acqua
1
0.8
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
-0.8
-1

Fig.13 :Total correlation within groups

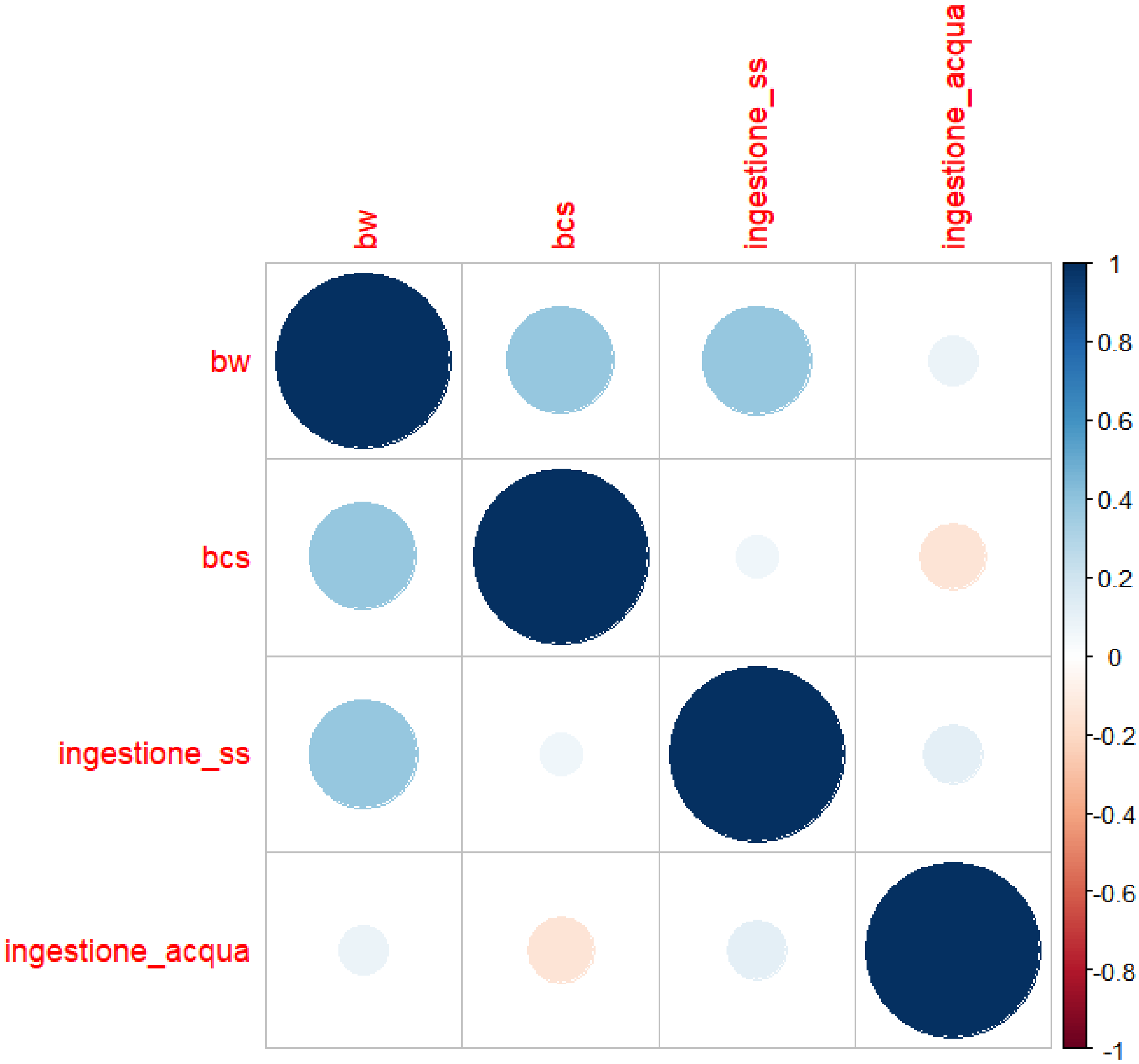


Table 7: Numerical values of correlations.

	bw - bcs	bw - ingestion_water	bw - ingestion_ss	bcs - ingestion_water	bcs - ingestion_ss	ingestion_water - ingestione_ss
a_n_c	0,62	0,16	0,42	-0,10	0,15	0,15
a_n_e	0,34	0,12	0,50	-0,20	0,12	0,11
b_e_c	0,22	-0,06	0,05	-0,21	0,08	0,08
b_e_e	0,44	0,03	0,38	-0,17	0,13	0,07
tot	0,38	0,08	0,38	-0,15	0,06	0,12

As evident from the overall trend, the two variables that show the highest dependence are liveweight and body condition score. Furthermore, BCS exhibits a negative correlation with water intake in all study cases. Generally, the correlations between the groups appear similar despite variations in diet and resistance. Consequently, it can be inferred that even animal groups with lower stress resistance demonstrate sufficient resilience to water restriction and environmental adversities.

5.2 Discussion

This study evidenced correlations between similar variables for all groups, except for those with low stress resistance and "normal" concentrate administration (b_e_c). In this case, the correlations assume a low value and close to zero, indicating a very weak relationship between the variables compared to that shown between the same in the other groups. This suggests that animals with less stress resistance, including in my study parasitic animals, exhibit a remarkable resilience, as even the lowest values do not differ much from the general trend.

Comparing the results with previous studies, it is observed that water restriction commonly leads to a loss of body weight as, in turn, it is closely linked to a decrease in food intake. Weight loss is then accentuated in conditions of high ambient temperature, especially during the summer. For example, sheep that were irrigated only in the evening recorded a reduction in body weight of 7.00% (in winter) and 11.00% (in summer). In other studies, during the water restriction, body weight reductions of up to 21.50% occurred, in the presence of very high temperatures (Akinmoladun et al., 2019).

Other trials with dry and lactating Awassi sheep reported weight reductions ranging from 0.84% to 26%. In fact, in this case, the study shows that the innovative system of water restriction every two days has not caused significant weight loss, even in the presence of temperatures up to 32 cox C. This confirms the rustic nature and good resistance of these animals. These results suggest a high adaptability of the Awassi breed to dehydration, with tolerance to water restriction regimes up to a month, losing only 16.8% of their body weight.

This ability to maintain food intake during periods of water shortage even in lactation, has been reported as an adaptive mechanism of the animal with the aim of ensuring an adequate intake of milk to feed infants.

The effect of the water restriction on the reduction of food intake is well known, and it has been observed that ewes in restriction mode reduce their food intake by up to 60%. Previous studies indicate that water restriction can cause metabolic changes even with a 50% reduction in food intake in just 3 days, affecting sheep's reproductive potential and reducing milk and meat production in sheep (Jaber et al.,2013).

Finally, considering the weight loss trend in my research, the results reflect what is reported in the present literature, confirming the body weight reduction as a consequence of water restriction, caused by both total body water loss and fat mobilization in the body. The attenuation of such loss in groups treated with vitamin C instead, suggests the beneficial potential of the vitamin itself in situations of water shortage, which is why it was also considered in my thesis paper as one of the most successful adaptive choices (Jaber et al., 2011).

6. CONCLUSION

The main focus is on understanding any significant differences between the two main macrogroups, namely animals fed with concentrates and those with lupins.

The study shows that the values of the correlations between the variables considered and the trend over the weeks in the different groups are surprisingly similar. This suggests that sheep selected for low strength are also resilient to heat stress and water restriction. In addition, the lupin feeding actually seems to mitigate the effects of the parasitic infestation, thus approaching the results of the two macrogroups. However, on average, the most resistant animals maintain higher values and trends.

Moreover, by taking blood samples during all the weeks of testing to evaluate metabolic parameters later, we expect to observe a level of cortisol, stress hormone, similar between different groups. Laboratory analyses will also be carried out on the quality of the meat to understand whether and to what extent stress has affected the yield at slaughter. However, these results will require additional time to be fully evaluated and are not part of this research thesis.

Finally, it is important to stress that studies like this are crucial to understanding and predicting the impact that climate change will have on farming systems. With increasing arid climates and increasing concern about water availability, it is essential to focus attention on animals with strong rustic and resilient characteristics, such as sheep and goats.

In many developing countries, such animals are already chosen as food and milk production resources because they have proven to withstand water scarcity well which, as in most of the literature reported, did not significantly affect the loss of appetite and weight of the animals themselves. Studies such as this show the adaptive capacity of these animals even when subjected to parasitosis, which in nature can be intercepted by grazing in pastures and which in effect represent a problem for the health and productivity of the animal.

In conclusion, our results indicate that the 40 selected Rasa Aragonese breed animals have demonstrated excellent resistance to environmental stress. These results are crucial for the future of livestock farming in a context of increasingly evident and urgent climate change.

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