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Effect of Thermal Stress on Livestock Production: Phenotypic and Genetic Analysis

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Chapter 1: Introduction

1. Impact of Climate Change on Livestock Production

The interest of research on adaptability to extreme heat and cold conditions has increased in the last few decades due to the global warming. The potential impact of the consequent changing of temperatures is expected to alter crop and livestock productions (Hatfield et al. 2008). This is even more worrying because of the increasing demand of products of animal origin caused by the growth of world population (Delgado et al. 2003).

As showed in figure 1, the total meat production increased from 96 M tons in 1970 to 321 million tons in 2017, respectively. Poultry and pig and showed the largest increase in this period (FAO 2017).

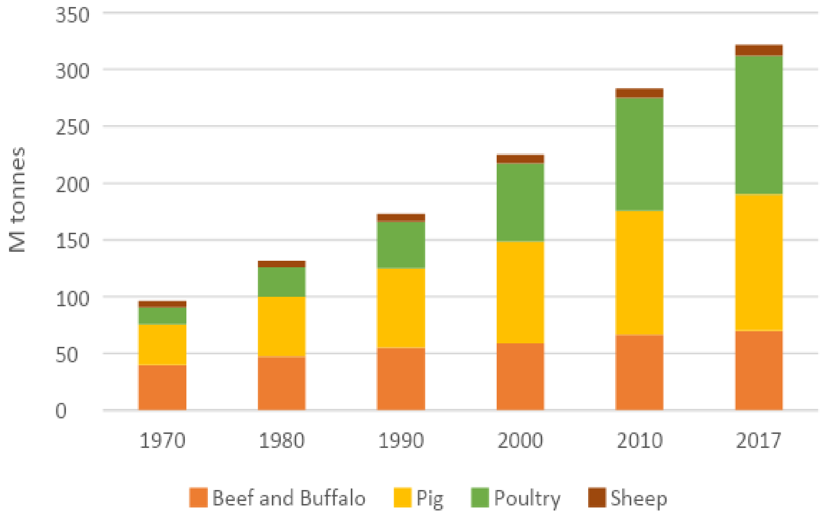

Figure 1. Evolution trend of meat production in developing and developed countries between 1970 and 2017 (FAO Statistics).

In the same way, Figure 2 reports the trend of milk consumption in developing and developed countries between 1970 and 2017. Of the world global milk production, in 2017, the largest amount came from cows (82%), whereas smaller amounts derived from buffalo (15%%), goat (2%), sheep (1%).

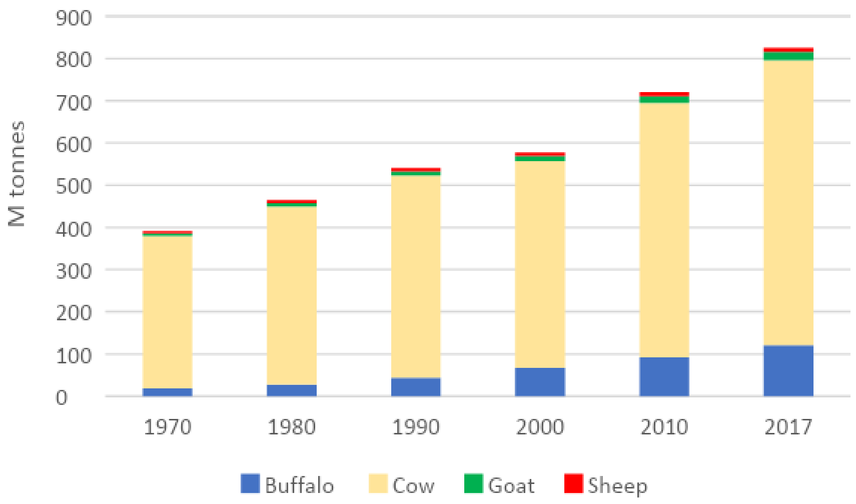

Figure 2. Evolution trend of milk production in developing and developed countries between 1980 and 2017 (FAO Statistics).

Considering the human population growth, the animal agricultural sector will need to expand the productivity in order to satisfy the increasing protein demand through the identification of the mechanisms by which extreme environmental conditions reduce animal productivity. It is widely known that the susceptibility of main livestock species (Cattle, pigs, sheep and goat species) to the thermal stress conditions can impact a variety of productive and functional traits (milk, growth, reproduction) and consequently result in a significant economic loss of the livestock industry. St-Pierre et al. (2003) reported that the annual losses due to heat stress for the US livestock industries averaged $897 million, $369 million, $299 million, and $128 million for dairy, beef, swine, and poultry industries, respectively.

Then, the comprehension of the mechanisms by which extreme environmental conditions affect the animal performance assume a considerable importance in developing the right strategies (i.e nutritional, managerial and genetic) to mitigate the impact of thermal stress conditions and optimize the animal productivity.

1.1 Thermoregulation and Heat Dissipation Mechanisms

The homeothermic animals that include the main livestock species are able to keep a constant body temperature through mechanisms of thermoregulation:

- *Conduction*, where the animal conducts heat to a cooler surface;
- *Convection*, where thermal currents leave the animal's body;
- *Radiation*, where the animal radiates heat to a cooler environment, such as the cool night air;
- *Evaporation*, where moisture is evaporated from the surface of the body (sweating) and from lungs (panting).

For the maintenance of their basic organic functions, all animals require thermal stability in normal conditions or in the presence of mild climate changes, and preferably, without the excessive use of temperature control systems. When animals are exposed for a long period to a moderately high temperature, they first present a phase of increasing basal temperature (*Tb)* that will then decline to a dynamic steady state corresponding to an 'acclimated' state (Collin et al., 2002; Renaudeau et al., 2010).

The relationship between environmental temperature (*Ta*) and the balance between heat production and heat loss is schematically reported in Figure 3. The thermoneutral zone (TNZ) is the interval of thermal environment, usually characterized by *Ta* over which heat production is relatively constant for a given energy intake. The lower and upper limits of the thermoneutral zone are called the lower (LCT) and the upper critical temperature (UCT), respectively.

When ambient temperatures either exceed or fall below the thermoneutral zone, the animal is forced into consistent functional, physiological and behavioral modifications (Nienaber and Hahn, 2007).

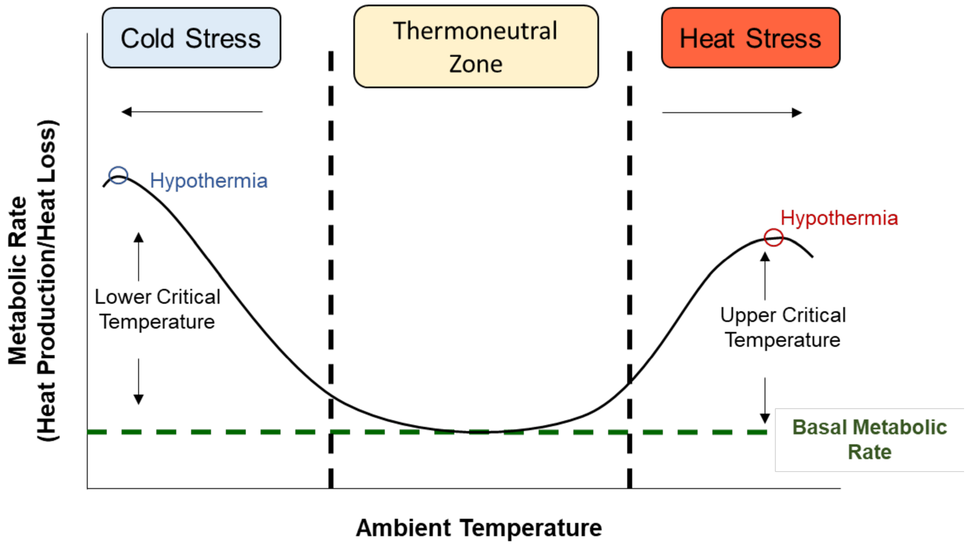

Figure 3. The thermoneutral zone lies between the upper and lower critical temperature.

In case of hyperthermia, animals produce more heat and start to release water (through sweating and/or breathing) as a consequence of the muscular work required for increasing respiratory and cardiac frequency. On the contrary, hypothermia occurs when heat loss is greater than its production and body temperature drops below the normal limit: both of these situations could result in the death of the animal (Bianca, 1976; Yousef, 1985).

Different environmental factors can determine the stressful conditions, producing a detrimental impact on production when environmental temperature and humidity fall outside the thermal comfort zone. Management precautions can produce differences in the effect of the same climatic conditions as well as the physiological stage (e.g. growth rate, stage of lactation, stage of pregnancy), the nutritional status, age and previous exposure to environmental conditions may increase or decrease the impact of thermal conditions (Hoffman, 2010).

Furthermore, the susceptibility to thermal stress conditions depends on breed, species, and the considered trait (Hoffman et al. 2010). The intensive selection on production has increased the degree of the antagonistic relationship between heat stress and productivity of some breeds compared to others less productive but more tolerant to wider range of temperatures (Ravagnolo and Misztal 2000; Carabaño et al.,2019).

In order to better understand the mechanisms of thermal stress adaptation is crucial the appreciation of the physiological and metabolic adjustments occurring under thermal stress conditions. Even if the impact of extreme temperatures in term of performance and well-being of animals are quite similar, the metabolic and physiological strategies required to mitigate the impact of extreme thermal conditions are different (Sejian et al. 2012).

The effects of heat stress and cold stress on livestock production traits (milk production, fed intake, body-weight gain) have been investigated in several studies. A brief list is reported in Table 1.

Table 1. The effects of heat stress (HS) and cold stress (CS) on livestock production variables.

Trait	Species	HS	Reference (HS)	CS	Reference (CS)
Milk production	Cattle	↓	Wheelock et al. (2010) Bernabucci et al. (2014) Bernabucci et al. (2015)	↓	Broucek et al. (1991) Johnson (1976) Soren (2012) Angrecka (2015)
	Sheep	↓	Finocchiaro et al. (2005) Sevi and Caroprese (2012) Peana et al. (2007)	– ↓	McBride & Christopherson (1984) Ramon et al (2016)
Feed intake	Cattle	↓	Johnson et al. (2015) O'Brien et al. (2010)	↑	Christopherson et al. (1979) Webster (1974) Young (1981)
	Pigs	↓	Johnson et al., (2013a) Johnson et al. (2013b) Johnson et al. (2014b) Pearce et al. (2013a) Baumgard et al. (2015)	↑	Lopez et al. (1991) Nyachoti et al. (2004)
	Sheep	↓	Marai et al. (2007)	↓	Ames and Brink (1977)
Body weight gain	Cattle	↓	Johnson et al. (2015a) Kadzere et al. (2002) West (2003)	↓	Soren (2012)
	Pigs	↓	Johnson et al., (2013) Johnson et al., (2014) Johnson et al. (2014b) Zumbach et al. (2008) Fragomeni et al. (2016)	↓	Ames and Brink (1977)
	Sheep	↓	Marai et al. (2007)	↑	

1.1.1 Lower Critical Temperature

The lower critical temperature (LCT) represents the temperature threshold below which the mechanisms of thermoregulation are aimed to preserve body heat (Young et al., 1983). Under the LCT the metabolic heat production starts to increase until a maximum point (summit metabolism) depending on the ambient conditions (Webster, 1977). When temperature decreases far below the LCT, the metabolism starts to change in order to increase the thermogenesis, leading heat production through mobilization of energy reserves (e.g. lipids and carbohydrates) (Webster, 1974).

An indicative metabolic adaptation strategy to cold temperatures is the increase of basal metabolic intensity which results in a series of complex physiological functions to maintain heat and prevent its loss (Smith et al., 1972; Young and Degen, 1980; Johnson et al. 2015). The increase in the metabolic demands of animals exposed to cold is usually reflected on an increase of appetite, rumination activity and rate of passage of digesta (Westra and Christopherson, 1976; Gonyou et al., 1979).

Species or breeds animal subjected for long periods (years) to prolonged cold period could have a series of genetic mutations that may modify their anatomical or morphological characteristics over time (Hoffman, 2010). Morphologically, the coat color, fat storage, body size and shape are the main adaptation strategies of livestock animals to low temperatures (Chedid et al. 2014). Animals with dark colors absorb more heat and increase more the thermal insulation than those with light coats (Asres, 2014). In a study aimed to determine the effect of breed on the heat tolerance of cattle, Brown-Brandl et al. (2006) reported that breeds with dark-hides (Angus) had higher respiration rates, panting scores, and surface temperatures than the breeds of cattle with light-colored hides (Charolais and Gelbvieh).

Also, fat tails and rump fat are considered an important energy reserve against cold stress during the winter and the migration period (Moradi, 2012). Fat deposition and the consequently higher cold tolerance, is also related to the age of animals: in early growth, fat and protein deposition occur simultaneously, but after a certain body weight protein accretion becomes negligible while fat gain becomes a large and constant fraction of body weight increase (Annison,1993). For this reason, younger animals show a lower ability to conserve heat due to the greater ratio between the surface and the body volume, and less presence of reserve substances (Le Dividich et al., 1991).

All these morphological changes would seem be associated with reduced blood flow in peripheral tissues due to cold, which would reduce the animals' surface contact with the external environment and therefore the heat dispersion itself (Webster, 1983).

Due to the increasing of global temperatures, the attention of literature is more oriented to the impact of heat- stress conditions on livestock performance production. Studies on the effect of low temperatures on animal performance are quite rare (Klain and Hannon 1969; Tamminga and Schrama, 1988). Among species, small ruminants (eg sheep and goat) are more adaptable than large ruminants to adverse environmental conditions due to greater body surfaces (Sevi et al., 2004).

Differences in cold susceptibility could be ascribed to the production aptitude of animals: breeding strategies to improve milk production in dairy cattle made them more sensible than beef cow to heat and consequently more tolerant to low temperatures (Bradford et al. 2016). Some studies reported that the winter feed requirements for beef cow increase by 30 to 70% because of the decreased digestion rate and the increased maintenance functions (Hironaka and Peters, 1969). Moreover, beef cows are usually farmed under extensive production systems and consequently they are more exposed to cold stress conditions (Young, 1983)

The geographical location and changing weather conditions determine the degree of cold stress of livestock animals (Young, 1981). The effect of cold stress on the animal is enhanced by the presence of wind, humidity, rain or snow. Wind and humidity have an additional effect because they reduce the external thermal insulation and increase the heat exchanges (McCarrick and Drennan, 1972). Further development of biological response functions is necessary to allow a greater application mitigation strategy across a large range of weather conditions (cold and hot scenario). Historically, a wind-chill index (WCI) was proposed by Siple and Passel (1945) combining ambient temperature (Ta) and wind speed (WS) to the time for freezing water. Considering the limitation of Temperature alone as environmental descriptor, a new equation has been also developed by Rothfusz (1990) and Tew et al., (2002). Only recently, Mader et al. (2010) developed a Comprehensive Climate Index (CCI) which adjust temperature effect incorporating major environmental components and better characterized both hot and cold conditions.

1.1.2 Upper Critical Temperature

The upper critical temperature (UCT) is the maximum body temperature that can be tolerated by an organism. Marai and Habeeb (2010) defined the heat stress as the condition in which animals activate physiological mechanisms in order to maintain the body's thermal balance. Under high environmental temperatures, the physiological and behavioral functions are compromised, resulting in less productive and reproductive performances of farm animals (West, 2003; Nardone et al., 2010).

In order to overcome the increase of body temperature, animals tend to increase the dispersion of energy through physiological or behavioral changes (Yasha et al. 2017). The first response to hyperthermia is the dilation of superficial blood vessels to limit thermal insulation and to promote heat loss by conduction, convection and radiation.

Change in heart rate, respiration rate and rectal temperature represent other thermoregulation mechanisms used by ruminant to maintain their body temperature (Kaushik et al. 2016). As a consequence, increase in sweating rate and in endocrine functions result in a reduced metabolic rate to overcome the heat metabolic production (Sejian et al. 2012). The rate of sweat secretion varies according to the part of the body of the animal (it is greater on the back) and according to the density of the sweat glands. Cattle have a gland at the base of each hair follicle on the whole body and their number per unit area is influenced by age, animal size and breed (McEwan Jenkinson and T. Nay. 1971; Nascimento et al., 2015). On the other hand, the totally lack of sweat glands in pigs hamper the regulation of their body temperature (Rhoads et al., 2013).

Some studies (Silanikove, 2000; Collier et al., 2008) reported different threshold of heat susceptibility according to species, breed and production level. Animals adapted to hot environments have a greater ability to reduce metabolic and endogenous heat production (Gaughan et al., 2009). Among species, sheep and goats are considerate to be the most adaptive to hot, adverse environmental conditions due to the different morphological characteristics and the less impact of genetic selection on productive performance (Sevi et al., 2004; Hoffman et al. 2010).

Although the higher thermal tolerance, exposure to high ambient temperatures has a negative impact on their production performance, including the nutritional and technological properties of milk (Sevi et al., 2002; Caroprese et al., 2012; Ramon et al., 2016). Different studies have been documented that an increase in rectal temperature occur from 38.97 and 43.66°C in goats kept for 6 hours in hot ambient temperature (Gupta 2013). On the contrary, cattle are the most susceptible to heat stress due to the high metabolic rate and poor water retention capabilities (Bernabucci et al. 2009).

1.2 Impact of Thermal stress on Animal production

The amount of research on the influence of thermal stress conditions on livestock productions depends on the investigated species and, in particular, on their importance on the world market production. The highest number of studies retrieved in literature deals with dairy cows, pigs and poultry, whereas fewer reports can be found for beef cattle, sheep and goats. As showed by Figure 4, many variables influence the impact of thermal stress on production traits, such as weight gain, disease incidence, reduced feed intake, reproductive efficiency, altered carcass composition and meat quality (Figure 4).

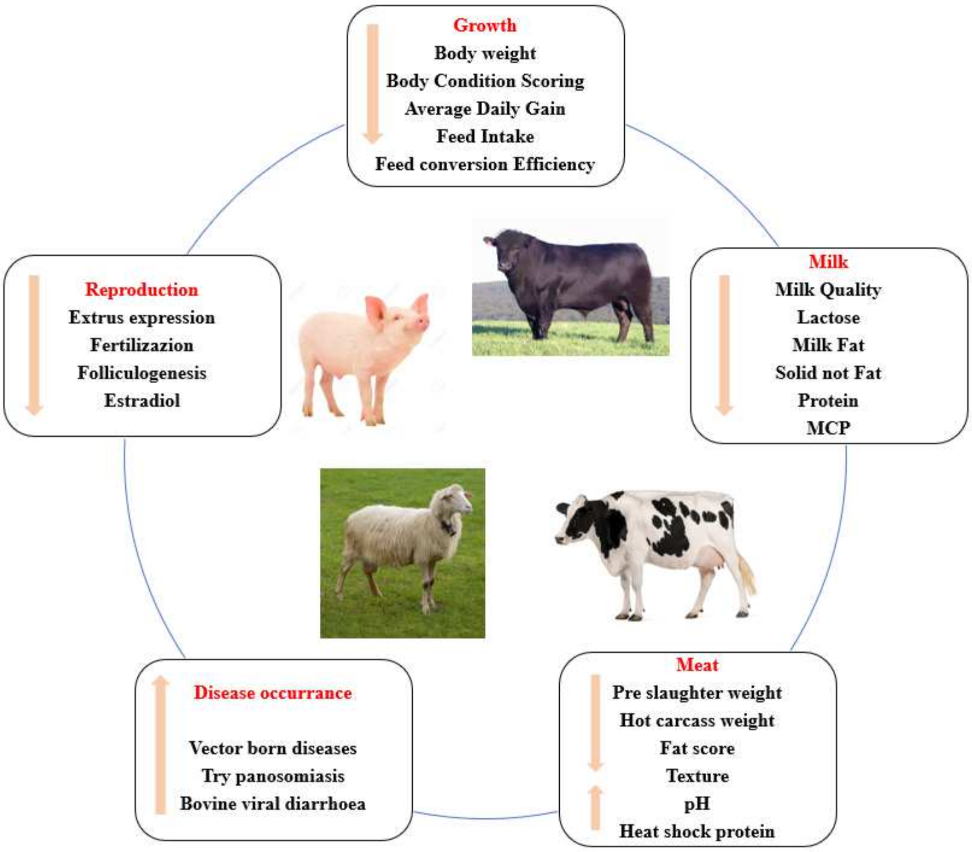

Figure 4. Climate Change and Livestock Production.

In the following paragraphs, the impact of adverse climatic conditions on traits of economic interest for some livestock species (dairy and beef cattle, sheep and pigs) will be discussed.

1.3 Impact of Thermal Stress on Lactation

1.3.1 Milk production

It is widely known that productivity and thermal tolerance are antagonistic traits (Ravagnolo and Misztal, 2000). Milk production of selected animals declines when metabolic heat production increases in conjunction with heat stress (Berman, 2005). Cattle are more prone to thermal stress than sheep and goats (Johnson et al., 1965), especially when the most productive breeds, such as Holstein Friesian, are considered (West., 2003).

In order to evaluate the simultaneous effect of temperature and humidity factors and to assess the risk of heat stress, a temperature–humidity index (THI) is commonly used. Many researches have highlighted that in dairy cattle milk production begins to decrease when the THI exceeds the value of 72 (Johnson, 1985; du Preez et al., 1990; Armstrong, 1994). In a study conducted on Holstein cattle, Ravagnolo et al. (2000) observed a constant protein and milk production trend until about 72 THI (0.2 kg/unit of THI for milk and 0.009 kg/unit of THI for protein, respectively). On the contrary, in climate-controlled high-yield cows, experiments indicated that milk yield starts to decrease at a THI of 68 (Zimbleman et al. 2009).

Several studies considered the consequences of climatic effects on the performance of dairy cattle (Ravagnolo et al., 2000; Bernabucci et al., 2010;). On the other hand, the negative impact of thermal stress on small ruminant’s milk production (sheep and goat) is more difficult to determine due to the strong seasonality of production (Segnalini et al. 2011; Peana et al., 2015; Finocchiaro et al., 2005). In Mediterranean basin, the overlap between summer season and last stage of lactation of ewes hampers the separation between the effect of climatic factors and other confounding factors (such as, stage of lactation, parity) on milk production and quality (Sevi et al., 2004; Ramon et al., 2016).

In Manchega sheep breed, a comfort region for milk yield between 11.5-21.0°C for average temperature and 19.1-29.6°C for maximum temperature, respectively, has been observed (Ramon et al., 2016). These values correspond to 10.3-18.0 for average THI and 13.9-22.0 for maximum THI, respectively. A similar study was conducted by Peana et al. (2007) in Sarda ewes: if maximum ambient temperatures exceed 21-24 °C, the milk yield can be reduced by 15%, and by 20% if minimum temperatures change from 9-12°C to 18-21°C, respectively. In the same way, Sarda sheep production performance can be reduced by 20% with THI moving from 60-65 to 72-75 (Peana et al., 2007). A higher threshold was observed on Comisana ewes by Sevi et al. (2001) who registered a reduction of milk yield after ewe exposure to temperatures over 35°C, even for short periods.

As reported below, in addition to thermal effects on production, thermal stress can also negatively affect the quality of milk products. Climate change has an important effect on organic milk and non-organic milk composition (Mariani et al., 1993, 1998). Likewise, this can directly influence the quality of cheese, especially those cheeses produced using raw and/or non-standardized milk.

1.3.2 Fat content

Recently, great interest has been directed towards milk fat and its fatty acid profile because of its effect on the technological, sensorial and nutritional properties of milk and dairy products (Chilliard et al., 2000; Hammami et al., 2015). Milk fat content is strongly influenced by weather conditions and seasons. The thresholds indicating the beginning of heat stress were found at THI values of 72 for fat yield in cattle (Bernabucci et al., 2014).

Generally, studies conducted on dairy cattle reported a decrease of fat percentage under hot and humid environments (Bouraoui et al., 2002; Kadzere et al., 2002; Zheng et al., 2009). Bernabucci et al. (2015) reported a significant decrease of milk fat of dairy cattle (3.20 g/100 g) during the summer season compared with the values observed in winter (3.80 g/100 g) and in spring (3.61 g/100 g). Alternatively, no significant differences were observed between cows in comfort environmental conditions and those under heat stress (Cowley et al., 2015).

Fat milk composition is also strongly related to depressed rumen conditions occurring during heat stress (Muna and Abdelatif, 1992). The increase energy demand for thermoregulation occurring in heat-stressed animals results in change of body condition score and a higher plasma NEFA concentrations that hampered the reconstitution of body fat but help the maintenance of milk fat content (Sevi et al. 2004). These changes determine the production of fatty acid biohydrogenation intermediates that alter milk fatty acid composition (Caroprese et al. 2011; Nudda et al. 2005). A study carried out on Comisana ewes has shown that solar radiation leads a change in the fat composition of sheep's milk, with a simultaneous increment of saturated fatty acids and decrease in unsaturated fat (Sevi et al., 2002). In particular, higher proportions of short chain and saturated fat acids (FA) have been observed in ewes exposed to solar radiation, due to the increased contents of lauric, caproic, myristic, capric and stearic acids (by 3-18%), and decreased contents of oleic, linoleic and linolenic acids (by 2-9%).

1.3.3 Protein and Casein Fraction

Milk proteins in milk are classified in two groups, defined by their chemical structure and physical characteristics: caseins (insoluble proteins), and whey proteins (soluble proteins). Among dairy milk characteristics, casein content and composition (which is constituted by several fractions, named αs1, αs2, β, κ, and γ caseins) are 2 important factors affecting the milk coagulation properties (Bertoni et al., 2001, 2005). Likewise, the role of the protein fraction composition and its seasonal changes is directly correlated to cheese production (Bittante et al., 2012). In generally, studies reported a decreased trend of protein content for higher temperatures in both small ruminants and cattle (Finocchiaro et al., 2005; Zheng et al., 2009; Hamzaoui et al., 2012). There is still insufficient information on changes in the protein fractions of milk (Bernabucci et al., 2002) and the relationship with the milk properties under thermal stress. Cowley et al. (2015) compared cows raised under heat-stress and comfortable conditions: an increase of αs1 casein and a decrease of αs2 casein for the cows subjected to heat stress was found. The other fractions did not exhibit any difference between those groups, in line with those reported by Bernabucci et al., (2015) who reported an increase in β-CN fragment concentrations (1-28) due to β-CN plasmin cleavage, associated with a decrease in milk yield in cows exposed to acute thermal stress. The β-CN peptide, derived from plasmin 1-28, leads to a reduction in milk secretion, because it acts in the apical membrane of mammary epithelial cells as a blocker of K+ channels (Silanikove et al., 2009).

1.3.4 Lactose content

Lactose is a milk component entirely synthesized in the udder from precursors. In cattle it is responsible for 60% of the osmotic-coupled water flow that produces milk volume (Silanikove et al., 2000). The synthesis of lactose determines the rate of daily milk production, so its content shows little variation during lactation (Vera et al., 2012).

Indeed, the concentration of lactose follows the pattern of milk yield: it is low at the beginning of lactation in colostrum, rises and then decreases during lactation; an opposite trend is showed by protein, caseins and fat content (Bencini and Pulina, 1997; Sevi et al., 2000; Nudda et al., 2003). Most of the studies that observed a significant influence of heat stress on lactose content regarded dairy cattle. Very little is reported about the impact of heat stress on lactose content of small ruminant (Hamzaoui et al. 2012). In dairy cattle, some authors found a decrease of lactose content, joint to milk yield as THI exceed 72 (Gaafar et al., 2011). Cowley et al. (2015) showed that the amount of lactose in milk does not vary significantly between cows maintained at the temperature-humidity index greater or less than 75. In the same way Abeni et al., (1993) stated that the lactose content is not affected by heat stress in cows.

1.3.5 Somatic Cells Content

Milk shows different types of somatic cells: epithelial cells, blood cells and cytoplasmic particles. Most of the blood cells are lymphocytes, macrophages and leukocytes that reach the mammary gland as a response to a local infection (Bergonier and Berthelot, 2003; Albenzio et al., 2011). The proportions of the different categories vary during the lactation and it depends also on the health status of the animal. The studies about this topic were carried out mostly on dairy cattle. In particular, several authors have documented a higher incidence of intramammary infections during summer (Smith et al., 1985; Waage et al., 1998; Cook et al., 2002). Reduced proliferation of peripheral blood mononucleate cells is present in dairy cattle under conditions of intense heat (THI>72) (Lacetera et al., 2005). Regarding seasonal variation, both Bernabucci et al. (2015) and Summer et al. (1999) reported an increase of somatic cell content in summer compared to winter and spring seasons. Indeed, the analyses of milk produced in northern Italy showed an increasing content of somatic cells in summer. Moreover, Abeni et al. (1993) found a constant somatic cell content of milk for values of THI>75.

The precise mechanisms underlying reduced cellular immune function in sheep under high temperatures remain undefined, due to the lack of information in literature. Ewes subjected to heat stress and reduced ventilation rate show increased cortisol levels; the increase in cortisol secretion can hinder their cellular immune response after the intradermal injection of mitogens and their IgG production after antigen injection (Sevi et al., 2002; Caroprese et al., 2012). In a similar study, Sevi et al., (2002) observed that the lymphocyte proliferation was higher in ewes exposed to solar radiation compared to shaded ewes and fed in the morning when temperatures are low. These results suggest that both protection from solar radiation and feeding animals in the afternoon can minimize the impact of thermal stress on ewe immune status by enhancing cellular immune response (Caroprese et al., 2009).

1.3.6 Milk Mineral Components and pH

The climatic conditions can also affect pH and mineral components of milk. A seasonal variation on mineral composition of cow's milk was observed by Mariani et al. (1993): they reported a lower content of milk ash and phosphorus during the summer period. Phosphorus and calcium are the main mineral compounds in milk, but also sodium, chloride, iodine, magnesium, and small amounts of iron are contained. Phosphorus has an important role in cheese making, and in some studies, a decrease in phosphorus was related to a worsening of enzymatic milk coagulation (Kume et al., 1987). The concentrations of Ca, P, Mg, Zn, Fe, and Cu are higher in sheep than in cows, but their presence is more variable depending on the diet and period of the year. Milk coagulation properties are worsened by the plasma mineral imbalance (due to a reduction of sodium, potassium, calcium and phosphorus and to an increase of chloride concentrations) that occurs during warmer periods (Kume et al., 1987). In addition, Abeni et al. (1993) stated that milk pH tends to increase when cows have been reared at THI values >75, leading to a deterioration in cheese production. A further study carried out on Comisana sheep showed that plasmin activity and calcium and phosphorus contents were higher in summer milk than in winter and spring milk (Sevi et al., 2001).

1.3.7 Milk Coagulation Properties

Many studies have focused their attention on milk coagulation properties (MCP) in cattle (Aleandri et al., 1989; Ikonen et al., 1999; De Marchi et al., 2008; Bonfatti et al., 2014). These properties are defined by three parameters: curd firmness (a30, mm), curd-firming time (k20, min) and rennet coagulation time (RCT, min), usually measured using mechanical or optical devices (Bittante et al., 2012). The significant effect of climatic conditions on MCP is related to the strong relationship between coagulation properties and casein content, titratable acidity, and mineral content. Great variability exists in milk coagulation properties, depending on ruminant species (cattle, buffalo, sheep, and goats), breed within species, and individuals (Calvo and Balcones, 2000; Bittante et al. 2012; Cecchinato et al., 2012). In dairy cattle, Mariani et al. (1994) reported an increased RCT in correspondence of the warmest months (negative effect), with the maximum values found in July (18.97 min) and August (19.42 min) and the minimum in January (15.73 min). However, the strong variability existing between different breeds shows that Italian Friesian cows have a constant lower value of a30 compared to Italian Brown, even if both breeds showed a decrease during summer (Malacarne et al., 2005). This is mainly due to genetic improvement, particularly on k-casein variant, acted in Italian Brown cattle breed. Moreover, the temperature-humidity index also has an impact on the K20, which increases when the index value is higher than 75 (Abeni et al., 1993). The effect of temperature and humidity index was also confirmed by a more recent study by Bernabucci et al., 2015 who found a significant increase of the A30 from summer (21.98 mm) to spring (33.60 mm) and to winter (35.93 mm).

As mentioned before most of these studies refer to cows, even though MCP of sheep and smaller ruminants are heritable and could bring benefits for the dairy industry (Puledda et al. 2016). Sevi et al, (2001) hypothesized that the reduction of Ca and P that occurs during the summer period could determine the deterioration of renneting parameters generally observed in summer milk of sheep. Likewise, the lactation period of sheep is often shortened during the summer months because dairy farmers stop to collect milk from farms due to a decrease in milk yield and the seasonal deterioration of coagulation.

The source of variation of MCP are different and ascribed to different factors such as the instrument analysis, stage of lactation, age and parity and polygenic variation (Bittante et al., 2012). Although the events occurring in summer could have a deleterious effect on MCP a further study are required in order to determine how much of this variability is explained by environmental climatic conditions.

1.4 Impact of Thermal Stress on Growth

1.4.1 Effect of Thermal Stress on Beef Cattle

The negative effects of thermal stress are more severe in dairy than in beef cattle (Baumgard et al. 2012). Moreover, the effect of thermal stress on milk yield at specific test days is more immediate and easier to measure than growth (Zumbach et al. 2008).

Normally, beef cattle are farmed under extensive conditions where the constant exposure to natural climatic factors makes it difficult to develop mitigation strategies. Furthermore, the high feedlot temperature (Ta often exceed 50°C) and high-grain diet often expose these animals to heat stress events (Brown-brandl et al. 2013). In USA, the economic losses due to heat stress were estimated for $370 million (St-Pierre et al.2003).

Meat quality of beef cattle as well as organoleptic characteristics, pre-slaughter and post-slaughter processing are also influenced by environmental conditions (Spehar et al., 2008). The intensification of animal industries and the growing meat demand from developing countries is increasing the interest of research on develop strategies able to decrease the vulnerability of beef cattle to heat stress (Gaughan et al. 2007a,b; Santana et al.,2014). This aspect is even most important if the market requirement to improve the production performance and the consequent preference of higher – level breeds by beef industry is considered (Hungerford et al., 2000). Most of these breeds (such as Angus cattle) are also morphologically more exposed to heat stress since black coat are more impacted by heat stress (Busby and Loy, 1997; Brown-Brandl et al., 2006).

Hammond et al. (1996) reported that Angus experience greater physiological effects of heat stress than Bos indicus and tropically adapted Bos Taurus breeds. Under field conditions, the identification of heat tolerant animals within different breed cannot be easily performed (Blackshaw, 1994; Hammond et al., 1996, 1998; Gaughan et al., 1999; Beatty et al.,2006).

The most common strategy used from farmers to improve the thermal tolerance is the cross between higher-level breeds with indigenous and most adaptative breeds (Gaughan et al. 2010). This is more common in beef cattle due to the less critical genetic antagonism between adaptation to high temperatures and high production.

Although the few studies conducted on beef cattle, body temperature and THI index are the most common methods used to assess the physiological response of an animal to the climatic environment (Gaughan et al., 2007a,b; Gaughan et al., 2010). Respiration rate has been shown to be a good indicator of heat stress (Brown-Brandl et al., 2005). Also, the THI and Heat load indices were implemented in some studies to investigate the possible genotype by environment (G×E) interaction through a use of different statistical models (Bradford et al., 2016; Laurenco et al., 2015).

These results suggested that further selection for cattle breed with effective thermoregulatory control will be needed in the future; however, as already stated for dairy cattle it may be difficult to combine the desirable traits of adaptation to high temperature environments with high production potential in cattle.

1.4.2 Effect of Thermal Stress on Pigs

Globally, pig is one of the most consumed farm animals (Figure 1). China is the biggest producer of pork meat (49%) while the European countries are the second largest in pig production (25%) and North American countries are third with 11% (FAOSTAT, 2017).

Heat stress economic losses are associated to growth and efficiency, health care costs, higher mortality and decrease of carcass quality, as widely documented in many studies (Baumgard et al., 2012; Rhoads et al., 2013). St-Pierre et al. (2003) estimated an economic loss from $299 to 316 million for the USA swine industry and most of these losses coincided with the time of year with the higher market price.

The studies conducted on swine have mostly considered the impact of heat stress rather than cold stress because most of the production occurs during (and following) the warm summer months (Ross et al., 2017; Zumbach et al., 2008). Compared to other species, pigs are those with a higher sensitivity to heat stress since the lack of functional sweat glands and a thick layer of subcutaneous adipose tissue (Ross et al., 2017; Baumgard and Rhoads, 2013).

However, the negative impact of heat stress has primarily been attributed to suppressed feed intake (Kemp and Verstegen, 1987). Renaudeau et at. (2012) reported a reduced fed efficiency for temperatures higher than 30°C in finishing pigs which results in a reduced growth rate. Another problem is related to the comprehension of how much of decrease in productivity is due to heat-induced reductions and how much is explained by the reduced feed intake. For instance, losses in body weight and the body condition score were similar in heat-stressed sows and their pair-fed thermal neutral counterparts, in contrast to what observed in growing pigs (Pearce et al. 2013; Sanz Fernandez et al. 2014).

Although conflicting results are reported in literature, thermal stress affects the pig carcass composition (Nienaber et al. 1987; Le Bellego et al. 2002). When the environmental conditions exceed the pig's thermal comfort zone, the energy expended to maintain body temperature is subtracted from the growth processes, compromising the global pig industry. This scenario represents a penalized aspect also for the genetic progress which has always been oriented towards increasing litter size and obtaining a leaner carcass (Fragomeni et al., 2017a, b; Zumbach et al., 2008). Several studies showed that the duration of heat stress reduces muscle mass and protein synthesis (Collin et al., 2001). In the same way, a greater fat deposition in hyper-thermic pigs was reported (Le Bellego et al., 2002). Sander et al. (2009) showed that, even if lipolytic enzyme activity is reduced under heat stress, the activity of the lipoprotein lipase of the adipose tissue is increased, suggesting that hyper-thermic animals have a greater storage capacity of intestinal and hepatic triglycerides.

The negative impact due to adverse thermal conditions can be alleviated by the management (e.g. cooling systems), which in any case require some costs. Furthermore, Renaudeau et al. (2012) observed that several years of studies (1970–2009) revealed that the effect of heat stress on feed intake and growth in pigs became more pronounced in recent years, supporting the hypothesis that genetic selection for growth and carcass traits increases pig thermal sensitivity.

1.5 Genetic Component and Thermal Tolerance

Predicted future climatic conditions, together with the current impact of climatic scenario on livestock production are increasing the need for selecting animals most adaptable to adverse environmental conditions (Carabaño et al. 2019).

Under stressful conditions genetic variations can occur, such as exposing new genetic variants, activation of different genes due to changing environment (Hoffmann and Hercus, 2000; Gibson and Dworkin 2004; Pfennig et al., 2010). Even if the genetic basis of heat dissipation is still poorly understood, it is necessary to propose new breeding programs to improve thermal tolerance. As pointed before, the strong antagonism between production traits and thermal tolerance makes it difficult find the right balance between high production performance and high thermal tolerance (Ravagnolo et al. 2000; Carabaño et al. 2019).

For meat production, the most common approach to improve heat tolerance is the crossbreeding between high-performance breeds and breeds that are locally adapted and more resistant to extreme climates. The same strategy is not employed in dairy breeds (e.g. Holstein) because the crossbreds would have reduced milk yield (McDowell et al., 1996; Carabaño et al., 2019).

In order to identify tolerant and sensitive animals without compromising the production performance, several studies have used different genetic tools to study the genetic basis of thermal stress (Bohlouli et al. 2017; Sanchez et al. 2009; Macciotta et al., 2017). In fact, in spite of the importance of resistance to extremal environmental condition, measurement of this effect and other stressors is really difficult. Most of the studies conducted on heat stress estimate the genetic value of heat tolerant animals collecting direct measurements on animals, such as physiological traits of rectal temperature, respiration rate and heart rate. These traits are very sensitive indicators of thermal stress, but they are really expensive to be collect and they are not available on a large scale for selection program. Alternatively, the same purpose has been achieved by evaluating the productive response of animals through reaction norm models implemented by using random regression (Bernabucci et al., 2010; Biffani et al. 2016; Bradford et al., 2016; Bohlouli et al., 2017). The first method to select for heat stressed animals was suggested by Misztal (1999) based on the estimation of the individual productive response to increasing heat loads (norm of reaction to changes in the environmental temperature). The same approach has been adopted in several studies (Ravagnolo and Misztal, 2000, 2002; Freitas et al., 2006) in order to estimate genetic parameters for both yield and reproduction traits under the influence of heat stress. The advantage of this approach is that there are no additional costs to the preexisting milk registration schemes. The disadvantage is that the current information on milk registration does not seem to fully capture the production response to high heat loads.

The deepening on the influence that genetic component has on the performance traits has been the subject of several studies (Ravagnolo and Miztal, 2002; Bohlouli et al., 2013; Carabaño et al., 2014; Brügemann et al., 2011). Another issue related to these studies is the detection of the greatest approach to better estimate heat load suffered by animals. Many authors used alternative definitions of indices that combine temperature, humidity and additional climatic factors considering productive/reproductive performance. Different THI definitions were reported in literature, depending on the extent of natural and artificial evaporative cooling (Freitas et al., 2006; Bohmanova et al., 2007). The various THI indices were also used considering the lag period between the date of recording of animal's performance and the date for which meteorological variables are available (Ravagnolo et al., 2000; Finocchiaro et al., 2005; Peana et al., 2017; Brügemann et al., 2012; Bertocchi et al., 2014). Indeed, the definition of the lag period better determines the response of a delay effect of climatic variables on animal's performance traits (Bernabucci et al., 2015; Carabaño et al. 2014). For this reason, in a study conducted on Valle del Belice sheep breed, Finocchiaro et al. (2005) used meteorological data referred to a period of four days before the day of milk recording, even if little differences were found. These results are in contrast with those reported by Ramon et al. (2016) who ascribe the greater effect on milk yield to weather conditions on the day of the test and on the two previous days, with a reduced effect of conditions in the previous days.

Recently, Sánchez et al. (2009) proposed a hierarchical model that could be useful to identify animals that are less sensitive to high temperatures. With this model, two different criteria were used for defining individual level of heat tolerance. The first was to find the individual thermotolerance threshold and the second was the estimation of the unknown thermotolerance threshold at which the performance trait start to drop.

Another approach was proposed by Macciotta et al. (2017) using the principal component values derived from eigen decomposition of covariance matrix in order to derive indicator variables of heat tolerance from milk production data in dairy cattle. The estimated variability across different animals under thermal stress condition reported by all of these studies showed that genetic selection for this trait is possible. However, it is also true that specific selection for resiliency would require a good definition of that trait and could take a long time, especially in cattle. Also, resilient animals may be less efficient in optimal environments. The correct approach to address climate change would be to continue current selection with a focus on using data from commercial environments and depend on improved management to address short-term challenges.

Objectives of the Thesis

The highest amount of studies about the impact of thermal stress found in literature provided a clear overview of the phenomena on dairy cows, pigs and poultry. Currently, the intensive selection on production has been increased the sensitivity of most productive species to thermal stress. Especially in dairy cattle there is a strong genetic antagonism between the level of production and heat tolerance joint to a high genomic/environmental (GxE) interaction between dairy production under cold, comfort and hot periods. Due to the less importance on the worldwide market, much less information exists in beef cattle, sheep and goats.

The main objective of this thesis was to evaluate the magnitude of thermal-impact on different traits of economic interest among three livestock species (sheep, beef cattle, and pigs) reared under different climatic-environments conditions and production systems. Furthermore, for beef cattle and pigs the genetic component of thermal response were estimated in order to explore the existence of GxE interactions across the wide thermal range endured by these animals. The thesis is organized in five main chapters.

The Chapter 1 is an introductive reviewed about the physiology mechanisms that regulate the thermal tolerance and a discussion on the main factors influencing productive traits.

The Chapter 2 aimed to assess the effects of Mediterranean climate conditions on the production performance of Sarda dairy sheep. Particularly, the effect of temperature and relative humidity was evaluated on milk composition and coagulation properties of these animals.

In chapter 3 has been evaluated the magnitude of thermal-impact on body weight in male calves of Avileña-Negra Ibérica local beef breed during fattening. As better described in this chapter, Avileña Negra Ibérica (ANI) beef breed is a local Spanish breed that is produced under an extensive production system. This general goal includes three more specific objectives (1) find the lag of time that better describes the interaction between thermal events and the animal performance; (2) define the thresholds, above or below which animals are adversely affected by the thermal conditions and therefore characterize the comfort region; (3) quantify weight variation due to the thermal stress.

In chapter 4 the genetic component of thermal response in male calves of the Spanish local breed ANI were investigated. In addition, it was explored the existence of genetic- environmental interactions (G x E) across the wide thermal range endured by these animals.

The objective of chapter 5 was to investigate the existence of a genomic - environmental interaction (G × E) for carcass quality traits (Loin depth, backfat and carcass average daily gain) of a reference population of commercial crossbreed pigs.

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Chapter 2: Effect of Thermal Humidity Index (THI) on Milk and Cheese related traits in Sarda Dairy Sheep

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Abstract

Milk produced by dairy sheep in Mediterranean Countries is almost entirely destined to cheese making. Milk composition and technological properties are therefore traits of great economic interest in dairy sheep farming. Temperatures in the Mediterranean basin can often exceed sheep thermoneutral zone, with negative effects on the performance of animals. In the present paper, the impact of temperature and humidity on milk composition and coagulation properties has been investigated. In this study, heat stress was modeled considering the effects of days in milk, month of lambing, parity, flock and the Temperature and Humidity index (THI) data provided from weather stations spread around the regional territory of Sardinia. Pearson correlations between the observed variables and the THI index reveled a generally low to moderate correlation, varying from 0.29 (Fat %) to -0.32 (Lactose %). Results of the statistical analysis revealed an association between THI and main components of milk (Fat, protein, casein, lactose, urea, NaCl). In particular, lactose content showed a clear decreasing pattern for increasing THI values. On the contrary, no significant effect of climatic variables was observed for milk yield and SCS, probably due to the overlapping between season and parity. The general worsening of the A30 and ILCY could be due to the slight reduction of casein concentration observed with the highest THI values (THImax class ≥ 70).

Keywords: Heat stress; Environment; Clotting properties; Rennet; Dairy sheep

1. Introduction

The impact of climate change is expected to increase the vulnerability of livestock farming systems in the Mediterranean area where dairy sheep industry represents an important economic sector. In many southern European countries, most of dairy sheep are semi-extensively farmed. Under these conditions, animal performances are strongly affected by environmental factors (Collier et al., 2005; Bernabucci et al., 2010). An example is represented by the dairy sheep industry of the Island of Sardinia (Italy), where about 3 million Sarda breed animals are farmed, sometimes in marginal areas characterized by adverse geographical and meteorological conditions (Macciotta et al., 1999; Peana et al., 2017). The farming system is seasonal and it is markedly influenced by the Mediterranean climate, which is characterized by a dry summer and a mild winter. Rainfalls occur mainly in autumn and spring. Animals are mainly fed on natural pastures, with supplementations given during autumn/winter season when herbage availability is low. The production and reproduction cycles are synchronized with the vegetative growth pattern. Lambings usually occur in autumn for mature and in late winter/spring for primiparous ewes, respectively. Lambs are nursed by their dams and weaned at about 1 month of age (Nudda et al., 2019). Thus, milking starts in December and drying-off occurs in July for both for yearlings and mature ewes (Carta et al., 2009; Cappio-Borlino et al., 1997). Milk production and cheese making are therefore concentrated during the winter – spring period (Piras et al., 2007). During late spring and early summer, when ewes are in late lactation, there is a marked decline in milk yield and quality (Pulina et al., 2006).

The assessment of climate effects on production traits is therefore of crucial importance for dairy sheep management and breeding under Mediterranean conditions, especially considering the relevant effect of heat stress on milk quality and cheese yield

(Finocchiaro et al. 2005; Bernabucci et al., 2010; Caroprese et al., 2011). Even though "adapted" sheep are considered one of the most resistant species to thermal stress (Silanikove, 2000a and 2000b; Khalifa et al., 2005), high temperatures determine a series of adaptive physiological responses which can negatively affect productive and reproductive performance, immune system response, and in general the physiological status of the animal. (Peana et al., 2005).

Several indices aimed at describing zones of thermal comfort and stress have been suggested in the last years to support management decisions related to animal performance, health, and well-being (Epstein and Moran, 2006).The most commonly used is the temperature humidity index (**THI**).Several studies on the effects of THI on milk production traits have been carried out in dairy cattle, which has been found to be very sensitive to heat stress (Ravagnolo et al., 2000, Bohmanova et al., 2005; Bernabucci et al., 2014).

Studies on the effects of climate on ewe production performance under Mediterranean conditions are rather limited and mainly focused on milk yield. A reduction of about 0.39 kg was reported for Sarda ewes when maximum and average daily temperatures increased from 21 to 24°C and from 15 to 21°C (Peana et al. 2007a), respectively. On Manchega ewes, the decline in milk yield ranged from 1 to 5 g/d per °C above an average daily temperature of 22°C (Ramón et al., 2016). A markedly higher temperature threshold was observed in Comisana ewes, where negative effects of heat on milk yield were detected only when maximum temperatures exceeded 35°C (Sevi et al., 2001). In terms of THI variation, a decrease by 20% in milk production was reported for Sarda ewes moving from 60–65 to 72–75 class (Peana et al. 2007a). A higher critical threshold, similar to values reported for dairy cattle, was found for Valle del Belice ewes by Finocchiaro et al. (2005) who observed an effect of heat stress on daily milk yield of 62.8 g per unit of THI when this index exceeded the value of 73.

Besides a possible effect of the breed, the variability of results obtained in the above reported studies could be due to the kind of climate variables used. The dissimilarity between results in different studies could be due to the difficult use of a comprehensive climate index that has application under a wide range of environmental conditions to estimate the amount of heat load in different studies. A further aspect to be considered when analyzing the response of the animal to climate variations is the acclimatization ability. The same author, showed that the lagged effects of thermal load (cold and heat stress) are already visible in the first hours after exposure and up to the next 2-3 days from the occurrence of the climatic event. After this period, a less negative effect of thermal load is observed as a consequence of the acclimatization of the individual.

Milk composition and technological properties are important traits for the dairy sheep industry, being almost all sheep milk processed into cheese (Pulina et al., 2018). High temperatures occurring during the summer period in Mediterranean areas increase the energy demand for thermoregulation. The lower feed intake observed under hot conditions and the subsequent possible use of fat and nitrogen body reserves, together with the increased milk pH may result in a worsening of milk coagulation proprieties (**MCP**) (Amaral-Phillips et al., 1993; Hammami et al., 2015). In particular, the reduction of the casein content and the changes in the casein composition may explain the loss in cheese yield and the alteration of MCP observed in cattle during summer (Calamari and Mariani, 1998). Although most of literature is focused on dairy cattle (Bernabucci et al., 1998; Cecchinato at al., 2012; Macciotta et al., 2012), negative effects of summer climate on milk coagulation properties have been reported also for dairy sheep (Sevi et al., 2001;

Sevi et al., 2004). In fact, lactations are often shortened to avoid the hot periods because of the small volumes of milk yielded and the deteriorated coagulating properties.

Thus, the aim of the present study was to assess the effects of climate conditions, and particularly of temperature and relative humidity, on milk yield, composition, and coagulation properties in Sarda dairy sheep.

2. Materials and Methods

2.1 Animals, milk sampling and laboratory analysis

The study was carried out in the period April-June 2014 on a sample of 686 ewes, officially registered in the Herdbook of the Sarda breed. Animals were farmed in 43 commercial flocks (16± 6.5 ewes sampled per flock) spread throughout the island of Sardinia, Italy.

Milking is usually performed by manually operated milking machines twice per day during the period from January to July (often at 0600 and at 1600 h). Thus, one individual milk sample of 100 ml per ewe was collected during the morning milking, added with preservative (bronopol, 62.5 µl/100 ml). Milk samples were stored at 4°C and analyzed within 24 h after collection. Chemical composition analysis was performed by the milk lab of the Sardinian Association of Animal Breeders within 24 h after collection. Fat and protein percentages, lactose percentage, pH, urea, NaCl, and freezing point were determined by MilkoScan^{TM} (Foss Electric, Denmark), and SCC via automated flow cytometry devices using the Fossomatic^{TM} (Foss Electric, Denmark). In this study SCC were expressed as Somatic Cell Score (**SCS**) (Ali and Shook, 1980). MCP were measured using the Formagraph instrument (Zannoni and Annibaldi, 1981) (Foss Electric A/S, Hillerød, Denmark): rennet coagulation time (**RCT**, min), curd-firming time (**K20**, min), and curd firmness (**A30**, mm). In addition, individual laboratory cheese yield (**ILCY**) was determined by the method described by Othmane et al. (2002). For details on milk composition analysis, MCP and ILCY see the paper of Manca et al. (2016).

2.2 Climate data

Climate data for the period April-June 2014 were provided by 20 meteorological stations of the Regional agrometeorological Service of Sardinia (ARPAS). Data for each flock were obtained from the closest station based on the availability of the ARPAS network for the considered period of study. In particular, the minimum, maximum and average distance of each weather station from the reference flock were 0.6 km, 49 km and 13.4 km, respectively. The chosen weather stations were those that better represented the geographical and pedo-morphological conditions of the area where flocks were located, considering the position, altitude and the distance from the sea.

The meteorological variables considered were the maximum daily temperature (T, °C) and minimum relative humidity (RH) registered 24-hour before the day of the milk test. Thus, a THI value was calculated for each hour of the 24 hours before the milk recording and then the average value was indicated as daily THI value (from 12:00 on the day of milking to 1:00 pm of the previous day).

Maximum values were considered in order to better estimate the influence of climate conditions on each production trait. The other two indices (average and minimum THI) did not add additional information and they were not taken into account in our study. Missing data were filled with T and RH up to a maximum of 5 days from milk test.

The temperature and humidity index (**THImax**) was calculated according to Kliber (1964) as:

$$\text{THI\_max} = \left(1.8\,Ta - \left(\left(1 - \frac{RH}{100}\right)(Ta - 14{,}3)\right)\right) + 32$$

[1]

where, Ta is the maximum daily temperature (in Celsius degrees), and RH is the relative humidity (%). Formula [1] was used in previous studies on Sarda breed under Mediterranean conditions (Peana et al., 2007a; 2007b; 2017).

2.3 *Statistical Analysis*

Data were analyzed with the following mixed linear model using the MIXED procedure of SAS (SAS, 2002).

$$Y = \mu + DIM + PAR + LMONTH + THI + F + e \qquad [2]$$

where *Y* is the response variable; μ is the overall mean*; DIM* the fixed effect of days in milking (5 levels: 1:> 48 d and ≤110 d; 2:>110 and ≤140 d; 3:>140 and ≤170 d; 4:>170 and ≤ 200 d; 5:> 200 d); *PAR* the fixed effect of parity (8 levels:1-7, >7); *LMONTH* is the fixed effect of lambing month (4 levels: 1= January; 2= February, March; 3= October, November; 4=December); THI is the fixed effect of the THImax divided in classes (5 levels: 1: <65 ; 2: 60 ≤ THI max< 67; 3: 67≤THImax<70; 4: 70 ≤ THImax <75 ; 5:THImax ≥ 75); F is the random effect of flock (43 levels) $F \sim N(0, \sigma^2_F)$; e is the random residual term $e \sim N(0, \sigma^2_e)$; THImax classes were created to balance the distribution of observations in each THImax class.

The percentage of variance explained by the flock was calculated as the ratio between $\sigma^2_{Flock} / (\sigma^2_{Flock} + \sigma^2_e)$ where σ^2_{Flock} and σ^2_e are the estimated flock and residual variances, respectively.

Pairwise comparisons for fixed effect were performed using the Tukey test.

3. Results and Discussion

3.1 Environmental conditions during the experimental period

Weather conditions in the island of Sardinia differ depending on the considered area. The surface of Sardinia is 24,100 km^2, 19,648 of which are classified as mountain or hill areas (Istat, 2017). Generally, the central part of the island is characterized by the highest summer temperatures (and consequently the THI index) whereas the lowest temperatures are typical of mountain areas, where frost may occasionally occur in March and April. In our study, 11%, 58% and 31% of flocks were located in mountain, hill and plains areas, respectively.

The THI max values showed an increase over the three- month period under study (Table 1), reaching the highest values in June. In a previous study carried out in Sardinia, Peana et al. (2017) reported a heat stress threshold for milk, fat and protein yield of THImax equal to 68. In the present study, the maximum daily THI was under such a threshold in the first month of the considered period.

Table 1. Basic statistics of meteorological data.

	April	May	June
Number Obs.[a]	136	316	234
THI max (mean)	63	68	74
THI max (min)	58	64	66
THI max (max)	69	76	80
THI max (SD[b])	3.57	3.23	3.44

[a] Number of observations of the climatic data for each month;
[b] Standard deviation;

3.2 Basics statistics

The basic statistics of milk yield, composition, and MCP across the whole experimental period are reported in Table 2. The average daily milk production, about 1.00 kg/d, is in agreement with previous reports on Sarda dairy Sheep (Pulina et al., 2006) but lower than the average (1.63 kg/d) reported by Pazzola et al., (2014). Also averages of pH, lactose, fat, and protein contents are similar to values reported by other authors for this breed (Pazzola et al., 2014; Nudda et al., 2015). Urea content observed in the present work was higher than the value reported by Pazzola et al., (2014) (31.81 mg / 100 ml), and lower than the one observed by Buccioni et al. (2015) (44.7 mg / 100 ml), respectively. The somatic cells score (SCS), was equal to 4.23, according to Nudda et al. (2003).

The RCT was in agreement with previous reports on the Sarda breed (Mele et al., 2006; Pirisi et al., 1999), whereas a significantly lower value (8.6 min) was observed by Pazzola et al. (2014). Average values for K20 and A30 agreed with previous reports in sheep (Pazzola et al., 2014) while higher values have been reported in cow's milk which generally has a K20 between 5 and 15 minutes (Bittante et al., 2012). Ferragina et al.,

(2017) obtained similar values of K20 (1,9 min) but lower values of A30 and RCT were reported (50.2 mm and 8.9 min, respectively).

The ILCY measured in the present study was in agreement with a report on Merino sheep (Corral et al., 2010) but it was higher than the value reported by Othmane et al., (2002) in Churra sheep (26.5%). Furthermore, the ILCY reported on Table 2 is markedly than the real cheese yield: for example, the Pecorino Romano which is the most important cheese produced by the milk of the Sarda breed, has an average yield of 17.30% (Pirisi et al., 2002). These differences could be mostly ascribed to the different conditions between experimental laboratory and the standard industrial transformation process. In fact, the determination of the yield in the laboratory involves the use of reduced amounts of milk and the forced purge through centrifugation while in the dairy industry the purging phase lasts about 12 hours depending on the type of cheese to be produced.

Table 2. Basic statistical description of milk yield and composition as well as cheese related traits.

Item	N[a]	Min	Mean	SD[d]	Max
Milk yield and composition					
Milk (Kg/d)	686	0.36	1.06	0.37	2.68
Fat (%)	685	2.55	5.87	1.30	11.06
Protein (%)	685	4.03	5.41	0.57	7.87
Casein (%)	685	3.05	4.21	0.47	6.10
Lactose (%)	685	3.78	4.88	0.26	5.58
pH	686	5.65	6.56	0.11	6.96
Urea (mg/100 ml)	685	9.9	40.3	12.3	72.7
NaCl (mg/100 ml)	685	64.3	137.3	30.9	281.6
SCS[b]	685	-0.10	4.23	2.08	10.7
Cheese-related traits[c]					
RCT (min)	686	2.37	13.40	4.56	29.37
A30 (mm)	686	2.94	55.57	12.50	74.48
K20 (min)	675	0.62	1.68	0.63	6.50
ILCY (%)	686	19.25	34.92	8.18	64.88

[a] Number of observations.
[b] Somatic Cell Score.
[c] RCT = rennet coagulation time; A30= curd firmness; K20 = curd firming time; ILCY =individual cheese yield.
[d] Standard deviation.

Pearson correlations between the observed variables (Table 3) were generally low, apart from the one between total proteins and caseins (0.99) percentages, being the latter the major contributors to milk protein content (Stocco et al., 2018). Also, the magnitude of negative correlations between variables and THImax was very low. Largest negative values were observed for lactose content, smallest positive correlation was observed for protein percentages, respectively. Low correlations between THImax and milk composition in sheep were reported also by Finocchiaro et al., (2005). They also observed that higher correlations could be obtained considering climate variables for longer periods than a single day. These suggestions agree with some reports on dairy cattle, where the highest unfavorable effects of heat on milk traits has been found when climate variables recorded some days before the day of the test were considered (West et al., 2003). However, in the study conducted by Ramón et al (2016) has been observed that the magnitude of environmental conditions effect on production losses was higher considering when the climate variable measured the day of the test and the day before the test was considered. In the same study was observed that the goodness of fit model really depended on the climatic variable used. In order to identify decays in production associated with cold or hot longer periods daily average temperature showed a better fit than those observed for daily maximum temperature which seemed to be useful to identify decays in production associated with abrupt changes of temperature. On the other hand, in a study conducted on Sarda ewes Peana et al., (2007) reported a reduction in milk yield when the mean THI of the day previous the day of control increased from 60-65 to 72-75, and milk yield increased by 10% when wind speed increased from 1.5-2.5 m/s to 2.5-4 m/s.

Thus, even if the temperature humidity index (THI) is the most commonly index used to evaluate the degree of heat stress in dairy cattle (NOAA, 1976; Armstrong, 1994) there are mixed opinions about this aspect (Dikmen et al., 2009; Ramon et al., 2016). In our study, although the complex relationship between weather and milk yield, in line with previous studies conducted on dairy ewes (Peana et., 2007; Finocchiaro et al.,2005) the temperature humidity index (THI) was chosen. Even if with some limitations, such as that it does not takes into account the evaluation of other atmospheric variables impacting on animals (i.e., wind, solar radiation, etc.), the THI is still widely considered a useful tool to underlying the mechanisms that are at the base of the effect of different meteorological on milk yield due to the combination between temperature and humidity variables (Bohmanova et al. 2007; Segnalini et al., 2011). Moreover, the THI index has been used by a lot of numerous previous studies in order to establish the threshold of thermal comfort zone in livestock animals, even if other factors (breed, nutrition, farm management) can modify the susceptibility of animals to environmental conditions.

Table 3. Pearson correlation coefficients and corresponding P-values for the observed correlation between the investigated variables.

Item	Milk yield and composition									Cheese-related traits[c]				
	Milk (Kg/d)	Fat (%)	Protein (%)	Casein (%)	Lactose (%)	pH	Urea (mg/ml)	NaCl (mg/1ml)	SCS[b]	RCT (min)	A30 (mm)	K20 (min)	ILCY (%)	THI[a] max
Milk (Kg/d)	*	-0.20	0.08	0.07	0.03	-0.07	0.17	-0.004	-0.06	-0.04	-0.09	0.07	-0.09	-0.09
		<.0001	0.04	0.07	0.43	0.05	<.0001	0.91	0.12	0.24	0.02	0.08	0.02	0.01
Fat (%)		*	0.43	0.46	-0.47	-0.13	-0.21	0.13	0.24	0.08	-0.13	0.05	0.53	0.29
			<.0001	<.0001	<.0001	0.00	<.0001	0.00	<.0001	0.04	0.00	0.18	<.0001	<.0001
Protein (%)			*	0.99	-0.40	-0.10	0.04	0.07	0.22	0.27	0.02	0.003	0.37	0.01
				<.0001	<.0001	0.01	0.26	0.05	<.0001	<.0001	0.57	0.94	<.0001	0.70
Casein (%)				*	-0.36	-0.10	0.04	0.01	0.20	0.26	0.03	-0.02	0.38	0.01
					<.0001	0.10	0.33	0.70	<.0001	<.0001	0.40	0.93	<.0001	0.74
Lactose (%)					*	0.11	0.17	-0.83	-0.41	-0.26	0.25	-0.24	-0.29	-0.32
						0.003	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
pH						*	-0.10	0.03	0.24	0.62	-0.15	0.30	0.17	-0.23
							0.01	0.41	<.0001	<.0001	0.00	<.0001	<.0001	<.0001
Urea (mg/ml)							*	-0.27	-0.20	-0.12	0.21	-0.14	-0.26	-0.25

	<.0001	<.0001	0.00	<.0001	0.00	<.0001	<.0001
NaCl(mg/1ml)	*	0.42 <.0001	0.29 <.0001	-0.24 <.0001	0.29 <.0001	0.17 <.0001	0.23 <.0001
SCS[b]		*	0.42 <.0001	-0.26 <.0001	0.31 <.0001	0.27 <.0001	0.02 <.0001
RCT (min)			*	-0.48 <.0001	0.63 <.0001	0.33 <.0001	-0.10 0.07
A30 (mm)				*	-0.68 <.0001	-0.30 <.0001	-0.11 <.0001
K20 (min)					*	0.27 <.0001	0.02 0.00
ILCY (%)						*	0.33 <.0001

[a] THI_{max} =Temperature – humidity index calculated from daily maximum temperature and minimum relative humidity;

[b] Somatic Cell Score

[c] RCT = rennet coagulation time; A30 = curd firmness; K20 = curd firming time; ILCY =individual cheese yield;

3.3 *Effects of Thermal Humidity Index (THI) on milk production and composition*

The statistical significance of the effects considered in the mixed model analysis is reported in Table 4. Parity affected significantly milk yield, variables related to udder health such as NaCl and SCS, and rennet coagulation time. The lactation stage had an effect of milk macro components. No significant effect of month or type of lambing was observed for any of the considered variables. THImax class affected significantly most of the considered traits (Table 4). However, contrary to what expected, no effect on milk yield and SCS was observed. These two traits did not show a defined trend among different THImax classes (Table 5), in contrast with those found by different authors on ewe's milk production performance (Sevi et al., 2001; Peana et al., 2007; Caroprese et al., 2011).

Results reported in this study can at least partly be ascribed to the confusion between the effects of season, lactation stage, and parity in the typical Mediterranean dairy sheep farming system. Being the productive cycle strictly seasonal, lactations start in late autumn and finish at the beginning of the summer. Thus, almost all ewes are in mid-late lactation when largest THImax values occurred (Table 1). A further confusion derives from the overlapping between season and parity: mature ewes lamb in autumn whereas yearlings lamb at the end of the winter. Studies conducted on dairy cattle usually reported that milk production starts to decline above 72 THI, with a marked decline around 76 to 78 THI (Ravagnolo et al., 2000; Bernabucci et al., 2010). However, Sevi et al., (2001) observed that milk production only decreased in ewes exposed to solar radiation for THI values greater than 80. In our work, such high THImax values were reached only during the month of June when the mean values of THImax was around 66 (Table 1). Furthermore, when temperatures begin to increase, ewes are in mid-late lactation and the advanced stage of lactation (in June) played an important role to cope with heat load due to the less milk production level and the consequently less metabolic heat production (Habeeb et al., 1997; Bernabucci et al., 2010). It is possible that the animals were more vulnerable to the high temperatures during the peak of lactation when lower values of THImax were reported (Table 1).

According to Sevi et al. (2004) most of the milk components were high significantly affected by THImax but also by the stage of lactation (Table 4). In particular, least squares means of fat percentage tended to increase across THImax classes, reaching the highest values for the fourth class (between 70 and 75). It should be pointed that the fat concentration depends significantly on the stage of lactation and milk yield declined with lactation stage, whereas fat and protein contents increased and lactose content decreased (Ploumi et al., 1998). Thus, the increase on fat percentage observed in our study could be explained by a concentration effect, typical of the latest stage of lactation in sheep when the higher temperatures occur. Working with East Friesian cross ewes also Morgan et al., (2006) observed a significantly lower fat percentage at both 21 days and 90 days of lactation. Voutsinas et al., (1988) reported a significantly increase on fat percentage in goat milk from the mid-lactation period to the last week 42 of lactation (2.7 % and 4.6 %, respectively) associated with an increase of protein content for the same analyzed period (3.0 % and 4.2%, respectively).

Protein and casein contents in this study exhibited a less defined trend than those observed for fat content (Table 5). Protein showed an irregular trend whereas casein tended to decrease in the highest THImax classes (THI >70). This pattern of variation makes difficult to compare our results with those of previous study which generally described an antagonistic relationship between protein content and thermal stress environmental conditions (Finocchiaro et al., 2005; Ravagnolo et al 2000; Ramon et al., 2016; Beux et al., 2017). As regard the casein percentage, the apparently decreasing trend is in line with those reported in a study conducted on heat-stressed versus comfortable cows by Cowley et al. (2015). The author observed an increase of αs1 casein and a decrease of αs2 casein for heat-stressed cows, reporting that cows exposed to heat stress produced milk with less protein than cows housed in a comfortable temperature environment. In Comisana ewes, Sevi et al., (2001) found that the exposition to solar radiation resulted in lower yields of casein and fat contents.

Reduction of milk protein under heat stress could be also ascribed to the specific downregulation of mammary protein synthetic activity joint to the increased protein turnover (Cowley et al., 2015). In fact, Bequette and Backwell (1997) stated heat stress conditions could results on a higher aminoacidic competition between structural proteins and casein.

The lack of statistical significance of the THImax effect on SCS is apparently in contrast to the increase of NaCl over THImax classes (Table 5).The similarity trend of SCS across different THImax classes and the NaCl increase could be the result of the reduction in mammary defence capacity due to thermal stress combined with the negative incidence of udder health problems in sheep due to the multiplication and survival of bacteria (Albenzio et al., 2002; Sevi et al., 2001). Consistent with these findings, Sevi et al., (2004) reported a detrimental effect of solar radiation on the hygienic quality of milk and a combined effect of time of feeding on immune function of Comisana ewes. The same study reported that no significant statistical difference was found for milk SCC across shaded ewes and ewes exposed to solar radiation but the greater bacterial load observed on ewes exposed to solar radiation resulted in higher numbers of pathogen microorganisms. Thus, the incidence of udder health problems due to the higher pathogen microorganism's incidence during the hot season could alter the sheep normal physiological functions increasing the NaCl content in udder observed in this work.

Quite a contrary trend was observed for lactose content in the current study (Table 5); The behavior of the lactose percentage across increasing THImax classes indicate a negative effect of the exposure to higher temperatures and humidity. These results are in agreement with previous reports in dairy cattle from different authors (Nardone et al. 1997; Shwartz et al., 2009). Lactose is the main osmotically active component in milk. Therefore, lactose trend is also in line with the close relationship that exists between the lactose synthesis and the amount of water drawn into milk that makes lactose a constant milk component throughout lactation (Pollott, 2004). In a concentrate supplementation study conducted on lactating Alpine goats by Soryal et al., (2004), lactose content showed higher values at the beginning of lactation and decreased in May and June.

The influence of meteorological conditions of different milking periods is also reflected on pasture quality (Abecia et al., 2017). The decreased urea content across different THImax classes (Table 5) is probably a consequence of the depression of feed intake and pasture availability, both in quantitative and qualitative terms, that occurs in the hot season. It is already known that in dairy sheep farming the herbage intake is influenced not only by the quantitative amount of feed intake but also by the qualitative characteristics of the pasture which is strongly influenced by the seasonal climatic changing of the year (Molle et al. 2008; Sitzia and Ruiz, 2016). Many studies conducted on heat-stressed dairy cattle, reported an important increase in plasma levels of urea nitrogen contents joint to the decline of protein content mentioned in literature (Rhoads et al., 2009; Wheelock et al., 2010; Cowley et al., 2015) probably explained by the alteration of net flux of amino- nitrogen under higher THImax values and a decline in rumen microbial synthesis and an increase in the utilization of amino acids for gluconeogenesis, both of which are related to lower energy and protein intakes (McGuire et al., 1989; Bernabucci et al., 1998). On the contrary, the behaviour of urea-nitrogen trend observed in this study (Table 5) showed a gradual decrease for increasing THImax levels (Table 5). This is in line with the seasonal pattern of grass growth that leads a higher protein content during the spring in Mediterranean areas. Milk urea is one of the good indicators of protein metabolism and intake (Cannas et al., 1998). The lower Urea content under higher THImax values could be the result of a progressively reduction of pasture availability during the summer when higher temperatures occurs. The grazed forages with higher protein content have been found to increase the protein content of milk but also lead an increase in blood urea levels (Cannas et al., 2004).

3.4 Effect of Thermal Humidity Index (THI) on Milk coagulation proprieties

Several studies have highlighted the effect of heat stress on the technological properties of milk in both sheep (Sevi et al., 2004; Jaramillo et al., 2008) and in cattle (Calamari et al., 1998; Bernabucci et al., 2015). In general, with higher temperatures and the progression of lactation there is a worsening of the attitude to coagulation of the milk, due to an increase in the RCT and a decrease in the consistency of the curd (Albenzio et al., 2004; Bernabucci et al., 2015). However, the effect of heat stress on MCP of small ruminants is less known. Even if heat stress is expected to affect the milk composition and its technological properties in ewes, the less importance of sheep regard to global milk production and the higher costs of phenotype recording results in a consequently lower selection intensity for high productivity explains the less heat stress attention from literature to this species.

In this study, the THImax factor significantly influenced the A30 and ILCY (Table 4). In particular, lower A30 for larger THImax values was observed, with significantly lower values occurring for THImax>67. The decreased trend of A30 showed in Table 4 is apparently in contrast with the increase of fat content described in the previous paragraph. In line with our study, Bernabucci et al., (2014) observed that the A30 in dairy cattle tended to be lower in summer, compared with winter and spring season but the decreased trend of A30 was also associated with a lower concentration of fat and protein in milk. However, our results seem confirm those reported in Sevi et al., (2004) on Comisana ewes. The author observed a marked reduction in A30 in milk collected during the summer season which was also associated with a slight increase in fat content. Similarly, a decrease of curd consistency was found by Bencini (2002) when high levels of protein and fat in milk were achieved.

Considering the really important role of the casein fraction on syneresis process (Amalfitano et al., 2019), the A30 trend observed in our work could also be associated with the apparently decrease in casein content under higher THImax classes (Pearse and Mickinlay, 1989). Pazzola et al., (2014) reported that the decreasing value of curd-firmness traits observed in Sarda dairy ewes was likely to be caused by curd syneresis and whey expulsion typical of the last stage of lactation when high temperatures occurred. A similar explanation of the A30 behavior in ewes during late- lactation stage has been done by Bittante et al. (2013) as the effect of curd syneresis causing whey expulsion was also demonstrated in Alpine sheep breeds (Bittante et al., 2014).

However, there are a controversial opinion regards the effect of different fractions of casein on MCP. Bernabucci et al., (2014) indicated that the worsening of MCP during the summer period was promoted by the reduction of casein concentration that determines consequences on pH, in line with those observed by Bittante et al., (2012). On the contrary, Remeuf and Hurtaud (1991) associated an increasing of MCP to the higher values of casein content.

The differences reported in results obtained between dairy cattle and ewes could be achieved to the strong interaction between the effect of lambing season (autumn vs winter) and the stage of lactation (Bittante et al., 2012). These two factors on milk ewe's quality results in a dramatic increase in RCT and a marked reduction in A30 in the milk collected during the hottest summer months (Sevi et al., 2004; Jaramillo et al., 2008; Martini et al., 2008; Bertocchi et al., 2014). In our study, no statistically significant influence of THImax effect was observed for rennet coagulation time (RCT, min) and curd-firming time (K20, min). This is in contrast with those reported in dairy cattle by Bernabucci et al., (2015) who found a worsening of MCP during hot conditions in summer season, mainly attributable to the reduction of protein concentration. However, according to previous studies carried out on dairy ewes (Sevi et al., 2004; Pazzola et al., 2014; Manca et al., 2016), RCT was influenced by the effect of parity. In fact, the coagulation behavior of milk is strongly related to the combined effect of climatic effect and seasonality of production cycle and stage of lactation of ewes (Pellegrini et al., 1997). Thus, effect of summer environmental conditions joint to nutritional factor related to lambing season could mask the THImax effect and the other factors on MCP.

A different and unclear trend was observed on ILCY (Table 5). The highest ILCY values corresponding to class 3 and class 4 of the THImax. These results are in contrast with those expected under higher temperature conditions (Sevi et al., 2004). However, studies regarded the changes in curd firmness during lactation showed that the increment of milk total solids reduced the draining capacity of the enzymatic curds increasing the ILCY of milk (Pellegrini et al., 1997, Remeuf and Raynal, 2001). As already reported in this study the progression of the lactation stage is in line with increasing temperatures in Mediterranean areas. Table 4 shows that the ILCY has been also strongly influenced by the stage of lactation (P = 0.007). Thus, considering that the complete coagulation of sheep milk is related to high contents of casein and fat (Sevi et al., 2004), the apparently increase of ILCY for highest THImax values could be associated to the slight increase of fat corresponded to the higher THImax classes found in our study (Table 5). Little information has been published regarding the effect of heat stress on the coagulation properties and ILCY. However, most of the studies regarded this topic stated that the cheese yield increased with the increase of the lactation stage whereas curd draining decreased (Jaramillo et al., 2008; Bittante et al., 2012).

The stronger effect of effect of lactation associated with the increase of ILCY at the mid and late stage of lactation stage, confirm also the results reported by Othmane et al., (2002) and Manca et al., (2016).

Moreover, the high values of ILCY obtained from the current study (higher than those normally reported by Pirisi et al., (2002) on industrial yield Pecorino Romano cheese) could be due to the micro-manufacturing experimental conditions that forced the draining process of a small amount of milk processing (Othmane et al., 2002; Manca et al., 2016).

Table 4. Significance of factors considered in the model.

	P[a]					
Item	Parity	DIM	Month of lambing	Type of lambing	THI max	r2F[b]
Milk (Kg/d)	<.0001**	0.215	0.972	0.259	0.230	0.55
Fat (%)	0.433	<.0001**	0.842	0.108	0.007**	0.34
Protein (%)	0.435	<.0001**	0.374	0.936	0.004**	0.12
Casein (%)	0.431	<.0001**	0.373	0.854	0.005**	0.14
Lactose (%)	0.106	0.006**	0.436	0.615	0.000**	0.15
pH	0.601	0.066	0.773	0.514	0.057**	0.33
Urea (mg/100ml)	0.761	0.146	0.048	0.735	0.032**	0.38
NaCl (mg/100ml)	0.005**	0.242	0.118	0.288	0.046*	0.06
SCS	0.030**	0.670	0.068	0.379	0.154	0.15
Cheese related traits[c]						
RCT (min)	0.024**	0.863	0.798	0.840	0.231	0.15
A30 (mm)	0.084	0.002**	0.397	0.717	0.003**	0.33
K20 (min)	0.082	0.867	0.582	0.574	0.347	0.15
ILCY (%)	0.778	0.007**	0.547	0.058	0.002**	0.27

[a] *P*-value

[b] The contribution of flock to the phenotypic variance

[3]RCT= rennet coagulation time; A30 = curd firmness; K20= curd firming time; ILCY =individual cheese yield;

** Represents the significance of THImax effect at level 0.01;

Table 5. Least square means and standard error of all variables for different classes of THI estimated in the mixed model analysis.

	THImax class[1]				
Item	1	2	3	4	5
Milk yield and composition					
Milk (Kg/d)	1.13 ±0.07	1.17 ± 0.06	1.03 ± 0.06	1.15 ± 0.06	1.07 ± 0.06
Fat (%)	5.35^{b} ± 0.24	5.73^{ab} ± 0.19	6.08^{a} ± 0.20	6.25^{a} ± 0.18	6.17^{a} ± 0.18
Protein (%)	5.47^{abc} ± 0.09	5.44^{ab} ± 0.07	5.20^{c} ± 0.07	5.48^{a} ± 0.07	5.25^{bc} ± 0.06
Casein (%)	4.25^{ab} ± 0.08	4.23^{ab} ± 0.06	4.04^{b} ± 0.06	4.07^{a} ± 0.06	4.07^{b} ± 0.06
Lactose (%)	4.93^{a} ± 0.05	4.89^{a} ± 0.03	4.88^{a} ± 0.04	4.74^{b} ± 0.03	4.75^{b} ± 0.03
pH	6.57 ± 0.02	6.58 ± 0.02	6.58 ± 0.02	6.54 ± 0.02	6.52 ± 0.02
Urea (mg/100ml)	44.24^{a} ± 2.48	39.94^{ab} ± 2.02	38.30^{ab} ± 2.10	36.07^{b} ± 1.91	35.76^{b} ±1.89
NaCl (mg/100ml)	134.1^{b} ± 5.30	142.8^{ab} ± 3.89	143.4^{ab} ±4.12	151.9^{a} ±3.92	151.7^{ab} ±3.76
SCS	3.83 ±0.40	4.39 ±0.31	4.28 ±0.32	4.95 ±0.29	4.39 ±0.29
Cheese-related traits[2]					
RCT (min)	14.76 ±0.89	13.60 ±0.68	13.26 ±0.72	14.41 ±0.66	12.96 ±0.65
A30 (mm)	54.37^{ab} ±2.64	59.72^{a} ±2.11	50.95^{b} ±2.21	51.95^{b} ±2.01	50.15^{b} ±1.99
K20 (min)	1.84 ±0.13	1.65 ±0.10	1.78 ±0.10	1.87 ±0.09	1.76 ±0.09
ILCY (%)	34.19^{ab} ±1.58	32.13^{b} ±1.24	37.22^{a} ±1.31	35.52^{ab} ±1.17	38.90^{a} ±1.16

[1] Classes of THI maximum; **1**: THI < 65; **2**: 65≤ THI< 67; **3:** 67 ≤ THI<70; **4:** 70< THI ≤ 75; **5:** THI >75.

[2] RCT= rennet coagulation time; A30 = curd firmness; K20 = curd firming time; ILCY =individual cheese yield.

$^{(a\text{-}b\text{-}c)}$ Means in a row with different superscripts differ (*P*<0.05).

4. Conclusions

In this work, milk production, composition, and technological properties recorded during spring- summer period were analyzed in order to investigate the impact of climate conditions in Sardinian region on those characters. The lactose was the milk component more strongly affected by the THImax effect. The general worsening of the MCP could be due to the slight reduction of protein concentration observed with the highest THImax values. The unclear pattern of protein observed during this period needs to be deeply investigated for better understanding the correlation with MCP during summer period. As observed in other studies on dairy sheep, the overlapping of climatic conditions, lactation stage, and parity made it difficult to properly assess the effect of climate on milk production traits in this species under Mediterranean semi-extensive and extensive farming conditions.

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Chapter 3: Characterization of the effect of thermal stress on the performance of the Avileña-Negra Ibérica calves at feedlot.

Abstract

Thermal stress is a research area of growing interest because of its effects on animal performance and animal production practices. This study aimed to analyze the impact of adverse weather conditions on the performance of male calves of Avileña-Negra Ibérica local beef breed during fattening. A total of 23,645 body weight records belonging to 5,876 animals collected between 2005 and 2017 were used. Meteorological data were collected for the same period from the station of Gotarrendura (Avila), the closest station to the feedlot in the national weather recording system. The lag effect of each weather descriptor – daily average (Tavg), maximum (Tmax) and minimum (Tmin) temperature, temperature-humidity index (THIavg), wind chill index (WCI) and the apparent temperature (CCI) – on weight gain was investigated for the recording date and up to 30 days before. Results showed that the impact of heat stress, if the animal were kept to a constant temperature, would be greater than that of cold stress in this breed. The comfort region ranged between 0.3°C and 25°C for daily average temperature (Tavg). The thermal load in the day of control (T0) proved to have more impact on the gain of weight at feedlot than thermal loads measured in previous days. This is particularly true for heat because of the large correlation between Tavg along the 30 d period. Thus, T0 could be interpreted as the accumulation of temperatures occurring during the whole period. The lost of weight of the ANI calves at feedlot out of the comfort region would be on average 1.9 kg per ºC of increase/decrease of Tave for heat and cold stress, respectively.

Keywords: Thermotolerance. Local beef cattle. Thermal lag. weight.

1. Introduction

The world's climate is changing at a rapidly increasing rate. The IPCC (2007) estimates that the global average surface temperature increased by about 0.6 °C during the twentieth century and the Mediterranean region will be one of areas significantly affected by a global warming. A recent study conducted by Segnalini et al., 2013 showed an increase of 4 units of Temperature-humidity index (THI) in Mediterranean countries during the summer periods of the decade 2041-2050. This climatic trend will increase the intensity and extent of the exposure to thermal stress particularly for species and animals under extensive conditions. Therefore, it seems legitimate to say that the growing impact of the climate change should attract research aiming to a better identification of adaptation strategies in response to expected future climatic conditions that are suitable for economic, social and managerial contexts.

Positive or negative deviations from the thermal comfort zone in feedlots for cattle can result in significant production losses (Belasco et al., 2015). St. Pierre et al., (2003) reported that across the United States, heat stress results in estimated total annual economic losses of $370 million in the beef industry.

The research related to climatic issues carried out worldwide deals to a larger extent with dairy cattle, which are very sensitive to heat stress (e.g., Ravagnolo et al., 2000; Aguilar et al., 2009; Hammami et al., 2013; Bernabucci et al., 2014; Carabaño et al., 2014). Most of the research about influence of weather on animal productivity has been devoted to study the effects of heat stress and there is very little information regarding the cold stress and the delayed effect of these uncomfortable climatic conditions on beef cattle. This is even more evident, if we take into account that, especially in Europe, the effects of adverse climate conditions on the quantitative and qualitative aspects of meat production are very little investigated in beef production system, resulting in a lack of information.

In many countries, cow-calf production is usually performed under extensive systems, which makes these animals particularly vulnerable, not only to extreme environmental conditions, but also to rapid climatic changes (Gaughan et al., 2008; Gaughan et al., 2010). However, these grazing animals have the ability to seek shade, water and air movement to cool themselves or seek for protection in cold periods to some extent trying to mitigate the effect of thermal stress. On the other hand, in many countries post-weaning periods occur in feedlots, where animals are confined and therefore very much influence by the quality of the design of feedlots. The Scientific Committee on Animal Health and Animal Welfare (SCAHAW, 2001) designated a thermal comfort range between 15 and 29°C, suggesting that the upper threshold temperature for beef cattle is 30 °C with relative humidity below 80% and 27 °C with relative humidity above 80 %. Out of that thermal comfort region, production losses are reported (see Mitloehner et al. 2001; Santana et al, 2015)

The Avileña Negra Ibérica (ANI) beef breed is a local Spanish breed that is produced under an extensive cow-calf production system with a post-weaning phase done in feedlots. The present study was conducted in the feedlot located in Castile - Leon region, Spain. This region is framed within the continentalized Mediterranean climate characterized by short, hot summers and cold and long winters. In this study, we aimed to evaluate the magnitude of thermal-impact on body weight in ANI calves at feedlot through the determination of the best descriptor of thermal stress (thermal load). This general goal can be broken down to three more specific objectives (1) find the lag of time that better describes the interaction between thermal events and the animal performance;

(2) define the thresholds, above or below which animals are adversely affected by the thermal conditions and therefore characterize the comfort region; (3) quantify weight variation due to the thermal stress

2. Materials and Methods

2.1 Animal data

The study was performed on weight data from 5,876 purebred animals of the Avileña Negra-Ibérica beef cattle breed. This breed, originally from the central highlands of the Spanish peninsula (Bos Taurus Ibericus) is characterized by the ability to take advantage of harsh environments (wide temperature range along the year and grazing in natural pasture of low quality for most part of the year) showing reasonable productive yields, high fertility rates and strong maternal qualities. Body weights were obtained in the commercial feedlot of Riocabado, a municipality located in the region of Castile and Leon, Spain. In this installation, animals are allocated in building partially shaded against radiation and well oriented to avoid the effect of air flows. The data set included 23,645 weight records collected during the period from September 2005 until October 2017, with a mean of 3 records (SD = 1.43) for each animal.

Because of its commercial attributes, most calves arrived at the feedlot just after weaning to be finished for another six to eight months. Other group of calves arrived after a period of fattening in the farm for later finishing in the common feedlot. Average age and weight at the beginning of the fattening period were 183 d and 314 kg, respectively. Final weight for eventual slaughter (around 16 months) was about 528 kg. Table 1 shows descriptive statistics of body weight data and the distribution of data according to the number of weights available per animal

Table 1. Data distribution according to the number of weights available per animal and summary statistics for weights and age of calves at entry and exit of the feedlot.

Data distribution			*Descriptive statistics*			
N^{w}[1]	Nr[1]	%	Stage	Parameter	Age (days)	Weight (Kg)
1	5876	100	At entry in the feedlot	Mean	266	314
2	5876	100		Std[2]	47	65
3	5400	92		Min	145	143
4	3751	64		Max	450	562
5	1828	31	At exit of the feedlot	Mean	437	527
6	704	12		Std	50.7	35
7	187	3		Min	312	355
8	23	0		Max	620	600

[1] ***N***^{w}: number of weights per animal over the period of study. ***Nr*** *number of animals*

[2] Std: Standard deviation

ANI calves in this particular feedlot are under an integrated production system where farmers participate from farms activities, feedlots, cutting plant and the benefit of sailing straight to consumers thus, there is a constant flow of animals along the year. To better understand patterns of exposure to thermal stress we built the Table2 where the distribution of months of entry and exit is shown. Based on the first and the last record date, we defined three subgroups associated to different thermal periods characterized by a similar climatic condition (cold, hot and comfort period). The majority of animals that entered the feedlot between November and April months ended their fattening phase around the summer season. This means that the animals spend most of their time into the feedlot over the coldest period of the year (cold subgroup). On the contrary, the animals that start from May until August leave the feedlot in October and November entering the feedlot during the hottest season (hot subgroup). We can see that most animals entered feedlot during summer time (46,6%), therefore heat stress period were mostly suffered for mostly younger animals; conversely, animals finishing the fattening period were going through cold period.

2.2 Weather data

Meteorological data was provided by the Spanish Agency for Meteorology (AEMET) and consisted of hourly values of air temperature (Ta,°C), wind speed (WS, m/s) and relative humidity (RH, %) collected during the period from 2005 and 2017. Records were extracted from 3 different weather stations, Avila, Gotarrendura and Revilla de Barajas, close to the feedlot location. The station of Gotarrendura was selected as the most representative of this study due to the closest proximity to the feedlot. Only when missing information in the Gotarrendura station was found with respect to dates of weighing in the feedlot, information from the other two stations was used, resulting in a dataset of 8,283 records. Based on hourly data we calculated the minimum (min), maximum (max) and average (avg) daily values of each climatic factor, which were merged with weight records. The temperature-humidity index (THI) was estimated considering that temperature and humidity influence much of the heat exchange impacts of warm and hot thermal environments. The daily average (THIavg), daily maximum (THImax) and the daily minimum (THImin) were calculated using the formula of Ravagnolo et al., 2000:

$$\mathrm{THI} = (1.8 * \mathrm{T} + 32) - \left(0.55 - (0.0055 * \mathrm{RH})\right) * (1.8 * \mathrm{T} - 26)$$

where T was the observed temperature in degrees Celsius, RH was the observed relative humidity on a 0 to 100 scale.

Obviously, effective environmental temperature may be temporarily cooler or warmer than the perceived temperature without compromising either the overall well-being or the productive efficiency of the animals (NRC, 1981). Wind speed is a factor that has a clear effect on the perception of temperature by animals. To account for this effect, the biometeorological index called the wind chill index (WCI) was also considered. This index allows us to assess the uncomfortable condition of cattle in relation to days with low temperature and high wind intensity. WCI index has long been studied to evaluate the wind effect in cold environments on exposed skin areas and represents a useful tool to express the cooling power of the wind. This is particularly important in this region to account for the freezing temperatures in winter periods that characterize this region of Spain.

In the present study, the WCI has been calculated using the formula proposed by Siple and Passel (1945):

$$\mathrm{WCI} = 33 - (33 - \mathrm{T}) - (0.474266 + (0.453843 * \sqrt{\mathrm{WS}}) - 0.0453843 * \mathrm{WS})$$

Where WS was the wind speed (in m·s−1) and T represents the air temperature in degrees Celsius, as in THI.

Finally, for each environmental variable, the specific adjustment to Ta was defined by the use of a comprehensive index for assessing environmental stress in animals, previously reported by Mader et al., (2014). The purpose of the CCI was to generate an indicator of the environmental conditions surrounding an animal. These mathematical relationships quantified how RAD, WS, and RH interact with Ta to produce an apparent temperature, which is represented by the CCI and adjusts Ta for the effects of respective environmental variables. Thus, equations were derived to allow adjustments to Ta due to the effects of RH, WS, and RAD. In our case the Ta variable was adjusted considering only the sub models derived for RH (Eq. [1]), WS (Eq. [2]) because animals were in a shaded feedlot, they were not exposed to RAD.

Equation [1] RH correction factor was:

$$e^{(0.00182*RH+1.8*10^{-5}*T_a*RH)}$$

$$\text{x}\ (0.000054*T_a^2+0.00192*\ T_a-0.0246)$$

$$\text{x}\ (RH-30)$$

Equation [2] WS correction factor was:

$$\left(\frac{-6.56}{e^{\left\{\langle\frac{1}{(2.26*WS+0.23)^{0.45}}\rangle*\langle 2.9+1.14*10^{-6}*WS^{2.5}-log_{0.3}(2.26*WS+0.23)^{-2}\rangle\right\}}}\right)-0.00566*WS^2+0.33$$

The CCI or apparent temperature is defined as Ta + Eq. [1] + Eq. [2].

Once indices were computed, each climatic variable for 30 days prior the date of weight recording were kept in order to examine the delayed effect (lag = 0,…,30) of heat and cold loads on weight of the animals..

Table 2 Distribution of month of entries and month of exit to describe patterns of expected exposure to thermal stress. The cold/hot/temperate periods are marked in blue/red/green.

Month of entry	***Month of exit***												***Total monthly animals entering into the feedlot***	***Histogram***
	Jan	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**		
Jan					3 4.2 %	12 16.7 %	27 37.5 %	20 27.8 %	8 11.1 %	1 1.4 %	1 1.4 %		72 1.23 %	
Feb				1 0.6 %	5 3.2 %	19 12.2 %	37 23.7 %	61 39.1 %	11 7.1 %	10 6.4 %	12 7.7 %		156 2.65 %	
Mar					3 1.5 %	17 8.7 %	23 11.8 %	97 49.7 %	33 16.9 %	12 6.2 %	10 5.1 %		195 3.32 %	
Apr					2 1.3 %	4 2.5 %	25 15.9 %	56 35.7 %	35 22.3 %	6 3.8 %	14 8.9 %	15 9.6 %	157 2.67 %	
May	21 5.5 %	9 2.3 %	7 1.8 %				12 3.1 %	31 8.1 %	88 23 %	102 26.6 %	72 18.8 %	41 10.7 %	383 6.52%	
Jun	117 13.1 %	61 6.8 %	42 4.7 %	33 3.7 %	2 0.2 %			11 1.2 %	89 9.9 %	153 17.1 %	245 27.4 %	142 15.9 %	895 15.23%	
Jul	99 14.2 %	117 16.7 %	100 14.3 %	45 6.4 %	11 1.6 %	3 0.4 %		5 0.7 %	33 4.7 %	60 8.6 %	121 17.3 %	105 15 %	699 11.90 %	
Aug	79 13.7 %	93 16.2 %	99 17.2 %	33 5.7 %	15 2.6 %	2 0.3 %			11 1.9 %	47 8.2 %	62 10.8 %	134 23.3 %	575 9.79 %	
Sep	104 14.5 %	92 12.8 %	152 21.2 %	102 14.2 %	85 11.9 %	47 6.6 %	14 2 %		1 0.1 %	14 2 %	31 4.3 %	75 10.5 %	717 12.20 %	
Oct	20 3.8 %	83 15.8 %	125 23.8 %	124 23.6 %	63 12 %	34 6.5 %	18 3.4 %	1 0.2 %		6 1.1 %	34 6.5 %	18 3.4 %	526 8.95 %	
Nov	13 2.4 %	19 3.4 %	58 10.5 %	114 20.7 %	114 20.7 %	86 15.6 %	102 18.5 %	44 8 %				2 0.4 %	552 9.39%	
Dec	7 0.7 %	8 0.8 %	31 3.3 %	113 11.9 %	143 15.1 %	181 19.1 %	233 24.6 %	185 19.5 %	47 5 %	1 0.1 %			949 16.15 %	
Total monthly animals leaving the feedlot	460 7.8 %	482 8.2 %	614 10.4 %	565 9.6 %	446 7.6 %	405 6.9 %	491 8.4 %	511 8.7 %	356 6.1 %	412 7 %	602 10.2 %	532 9.1 %	5876 100 %	

2.3 *Statistical Analysis*

Statistical analyses aimed at i) finding the lag between the weight observed in a given day and the day for which the thermal load exerts the largest influence on that weight ii) at defining the comfort zone of the animals during their period in the feedlot, and, iii) at quantifying the expected loss in weight due to heat or cold stress.

2.3.1 *Estimation of the Lag Value and Thermal Load Effect on weight*

The effect of weather variables and the lag between the day in which the weight is measured and the day in which the weather variables have the largest effect on the observed weight were jointly estimated using a lag non-linear model (DLNM) implemented in 'dlnm' package of R (Gasparrini, 2011; https://cran.rproject.org/web/packages/dlnm/index. html). This model was originally proposed in econometrics by Almon et al. (1965), and used by Armstrong (2006) and by Gasparrini (2014). This model constitutes a general framework applicable to linear or non-linear lagged relationships, allowing the definition of the relationship between a set of predictors (thermal load in this case) and a response variable (the weight of animals). This approach has been recently used with success to evaluate the effect of thermal load and lag on milk production in ewes (Ramon et al., 2017).

The dataset used in this study was the time series of individual weight recordings (N=23,645) and climate information for the date of recording and 30 days previous to this date. Weights were adjusted by 'noise' effects that could mask the relationship between weight and thermal load fitting the following model,

$$y_{jkli} = CG_j + GA_k + b_k \, age_l + e_{jkli} \qquad [1]$$

where, y_{jkli} is the weight record taken in ith animal, at age l nested to the kth group of age at entry to the feedlot (2 levels; age ≤ 247 and age > 247 days) in the jth contemporary group (CGj), defined by the combination of year and conventional season (j=1,…,47). Thus, in this model the effect of age on weight was assumed to change depending on the age of entry.

Four different analyses, one for each of the climatic variables described above, were conducted in order to better identify the effect of the lag and thermal load on weight. A period of 30 days prior to the day of weight recording was considered to study the lag effect.

In order to express the potentially nonlinear relationship between weight and thermal load and weight and lag, polynomials of 3rd and 4thdegrees were defined for thermal loads and lag, respectively. The orders of the polynomials were chosen because they provided the best fit of the model. Then, the DLMN approach provides a standard regression model to jointly estimate the parameters that define weights, varying along each of the two dimensions, lag and thermal load. This approach allowed then, to select the lag that best reflects the impact of each thermal load variable on changes in weight.

2.3.2 Definition of comfort regions

Once lags for each climate variable were established, the thresholds that define the comfort region for these variables in this population were obtained in a two-step procedure. A model to estimate the unstructured (with no assumption about the shape of the response function) response of weight to the thermal load in the day of the optimal lag was fitted as follows:

$$y_{ijkml} = CG_j + GA_k + b_k\, age_l + w_m + a_i + e_{ijkml} \qquad [2]$$

Model [2] included the CGj and GAk effects as in [1] and also the thermal load class wm, defined as the integer part of the heat load (m=1,…, X/Y/Z/ for Ta/THI/WCI/CCI, the random effect of the animal producing the weight ai ~ N(0,σ2a) (i=1,…,5876), and the random error, eijkml ~ N(0,σ2e). This model was solved using the "nlme" package of R for repeated-measures designs (https://cran.r-project.org/web/packages/nlme/nlme.pdf). In a second step, least square means for the thermal load effect (wm) obtained from [2] were then used as dependent variables to estimate the thermal thresholds through the methodology proposed by Muggeo et al. (2003). This method, implemented in the "segmented" package of R, was used to identify the heat and/or cold thresholds that represent points of change (decrease) in the observed response of weight to thermal load and to quantify this effect on growth of the animals. As reported in the reference manual (https://cran.r-project.org/web/packages/segmented/segmented.pdf), "the library is aimed to estimate linear and generalized linear models having one or more segmented relationships in the linear predictor. Estimates of the slopes and breakpoints are provided along with standard errors". The method is an iterative procedure that needs a priori definition of the number of breakpoints and starting values for them. In our case, the number of breakpoints was set to two, each of them representing the threshold for cold and heat stress respectively, thus defining the thermal neutral zone, out of which growth begins to decrease because of the effect of thermal streess.

3. Results

3.1 Characterization of Climatic Conditions

Table 3 shows a summary of monthly mean temperatures for the period of study in the region of Castile and Leon. Most of this part of Spain has a continentalized mediterranean climate with long cold winters and short warm summers. The coldest periods of winter are associated with continental polar fronts that generate an average daily maximum and minimum temperatures around 4 and -1°C, respectively. The most extreme minimum values vary between -11° and -13°C during the period from December to March. The temperature (Ta) starts to increase during the spring reaching the maximum value in July with 22°C of average monthly Tavg and 30ºC of average monthly Tmax with peaks above 38ºC.

For the relative humidity (RH), the annual trend is opposite to that of the temperature, with high RH in winter and low in summer, being the variation between both seasons quite large. Thus, the average annual humidity is around 64%, with a great oscillation between the cold months (80%) and the dry warm period (44 %). The low relative humidity observed during the hot period could decrease the thermal perception by the animals. The trends observed for all THI indices were very close to those estimated for corresponding temperatures, with low values in winter and high during the summer. On average, the windiest days were seen during the February – March period whereas the least windy month was September though the feedlot is particularly well design to avoid the effects of airflow on growing calves.

The comparison among weather variables was performed in terms of Pearson’s correlation coefficients. Table 4 showed all correlations coefficients among all climatic descriptors and the indices, WCI and CCI. All correlations are higher or equal to 0.80

Correlations between temperatures and the corresponding THI, WCI and CCI are particularly high (0.97 to 1.0). The block average correlations for temperatures, THI, WCI and CCI were all very high and of similar magnitude. These correlations were 0.940, 0.947, 0.941 and 0.931, respectively. The four blocks have similar average values with the rest. Tavg was high correlated with the other climatic varibles (between 0.94 and 1.00).

Table 3 Mean of climatic variables for each month over the years 2005 to 2017

Climate Variable[1]	Month of year											
	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
T_{avg}(°C)	4	5	7	11	15	20	22	21	19	13	7	5
T_{max}(°C)	8	10	13	17	22	27	30	29	26	19	12	10
T_{min}(°C)	-1	1	1	4	8	11	14	13	11	7	3	1
THI_{avg}	42	45	49	53	59	63	66	65	62	56	48	45
THI_{max}	47	50	56	62	70	77	81	79	75	66	54	50
THI_{min}	40	42	47	51	57	64	68	67	62	56	46	41
RH_{avg}(%)	82	77	68	68	59	52	44	47	53	67	80	80
RH_{max}(%)	95	93	88	88	84	77	70	72	77	86	94	93
RH_{min}(%)	64	57	46	46	36	31	24	26	32	46	61	62
WS_{avg} (m/s)	3	4	4	3	3	3	3	3	2	3	3	3
WS_{max}(m/s)	6	7	7	6	6	6	6	6	5	5	6	5
WS_{min}(m/s)	1	1	1	1	1	0	0	0	0	0	1	1
WCI_{avg}(°C)	3	3	6	9	13	18	21	21	17	12	6	3
WCI_{max}(°C)	8	9	12	15	20	26	29	29	24	18	11	8
WCI_{min}(°C)	-1	-1	0	3	6	10	12	12	9	6	2	-1
CCI_{avg}[2]	-1	-1	2	6	11	16	19	19	15	10	3	0
CCI_{max}[2]	2	2	6	10	16	23	27	26	22	15	6	3
CCI_{min}[2]	1	1	3	6	9	13	16	16	13	10	4	1

[1] $T_{avg/max/min}$ =daily average/maximum/minimum temperature; $RH_{avg/max/min}$=daily average/maximum/minimum relative humidity; THI $_{avg/max/min}$ = daily average/maximum/minimum temperature – humidity index; $WS_{avg/max/min}$ =daily average/maximum/minimum wind speed; WCI $_{avg/max/min}$ = daily average/maximum/minimum wind chill index; CCI $_{avg/max/min}$ = daily average/maximum/minimum comprehensive climate index;

[2] From Ta + Eq. [1] + [2]

Table 4 Pearson correlation coefficients between the climatic variables and indices

	Climate Variable[1]											
	T_{avg}	T_{max}	T_{min}	THI_{avg}	THI_{max}	THI_{min}	WCI_{avg}	WCI_{max}	WCI_{min}	CCI_{avg}	CCI_{max}	CCI_{min}
T_{avg}		0.97	0.94	0.99	0.96	0.96	1.00	0.97	0.94	0.97	0.94	0.94
T_{max}	0.97		0.85	0.97	0.99	0.91	0.98	1.00	0.85	0.97	0.99	0.89
T_{min}	0.94	0.85		0.93	0.84	0.97	0.94	0.85	1.00	0.87	0.80	0.96
THI_{avg}	0.99	0.97	0.93		0.97	0.97	0.99	0.97	0.93	0.96	0.95	0.94
THI_{max}	0.96	0.99	0.84	0.97		0.90	0.97	0.99	0.84	0.97	0.99	0.88
THI_{min}	0.96	0.91	0.97	0.97	0.90		0.96	0.90	0.98	0.91	0.87	0.96
WCI_{avg}	1.00	0.98	0.94	0.99	0.97	0.96		0.97	0.94	0.97	0.95	0.94
WCI_{max}	0.97	1.00	0.85	0.97	0.99	0.90	0.97		0.85	0.97	0.99	0.89
WCI_{min}	0.94	0.85	1.00	0.93	0.84	0.98	0.94	0.85		0.88	0.81	0.97
CCI_{avg}	0.97	0.97	0.87	0.96	0.97	0.91	0.97	0.97	0.88		0.98	0.93
CCI_{max}	0.94	0.99	0.80	0.95	0.99	0.87	0.95	0.99	0.81	0.98		0.86
CCI_{min}	0.94	0.89	0.96	0.94	0.88	0.96	0.94	0.89	0.97	0.93	0.86	

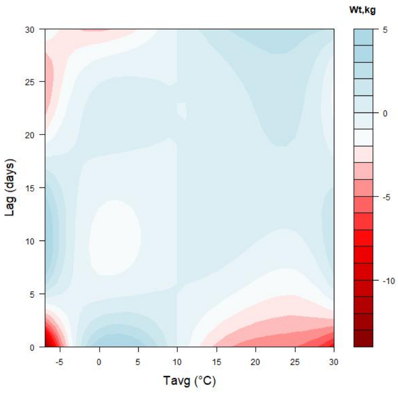
[1] $T_{avg/max/min}$ =daily average/maximum/minimum temperature; $RH_{avg/max/min}$=daily average/maximum/minimum relative humidity; THI $_{avg/max/min}$ = daily average/maximum/minimum temperature – humidity index; $WS_{avg/max/min}$ =daily average/maximum/minimum wind speed; WCI $_{avg/max/min}$ = daily average/maximum/minimum wind chill index; CCI $_{avg/max/min}$ = daily average/maximum/minimum comprehensive climate index;

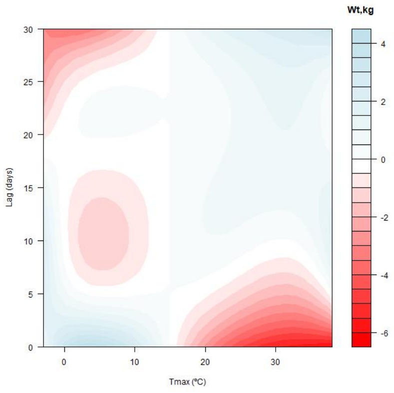
3.2 Estimation of Lag effect of Thermal Load Effect on weight

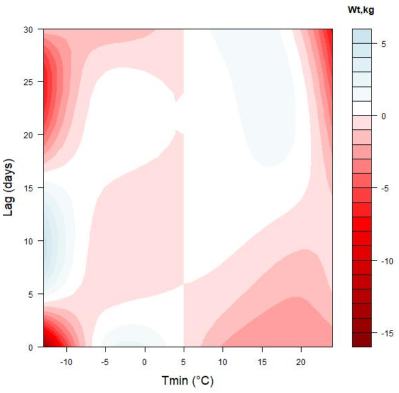
The effect that thermal load measured in the day of weight recording and the 30 previous days have on weights was evaluated using a joint analysis that included the effect of a certain predictor of thermal load and the lagged effect of such predictor, simultaneously. The response prediction is bi-dimensional. Figure 1 shows the contour plots obtained from these analyses. In each plot, the relationship between climate and weight throughout a period of 30 days is presented. Due to the high correlation observed among all the climate variables considered, a similar trend was obtained for all of them. Figure 1 shows that under hot conditions, largest negative effects were observed for the day of recording and previous days, decreasing this effect as we move away from this day. The lag-response association estimated for moderate and the highest values of the average and maximum climate variables (Tavg, Tmax, WCIavg, WCImax, THIavg, THImax, CCIavg, CCImax,) indicated that the strongest effect occurred at lag 0 and then declined rapidly, with a relative strong effect retained during the three – four days before the recording. This is even more evident considering the THI index. Weight losses were observed for a range of 60 – 75 and 70- 80 units of THIavg and THImax, respectively. For the cold side of the thermal load, the day of recording and days 20-30 showed the largest negative effects on weights for average and minimum values of the temperature and indices. For maximum values, only negative effects were observed for lags between 20 and 30 days. Only in case of values below to -10 °C for Tmin and WCImin and below 25 units of THImin the losses of weight occurred at lag 0. Then, with increasing lag time, the risk of cold temperatures increased gradually, whereas the increase in risk of hot temperatures was much smaller. Furthermore, for moderately low values of each climatic variable (between - 5 and 0°C corresponding to 30 – 40 of THI values) the effect of exposure to moderate cold had an impact at 5 and 15 days of exposure.

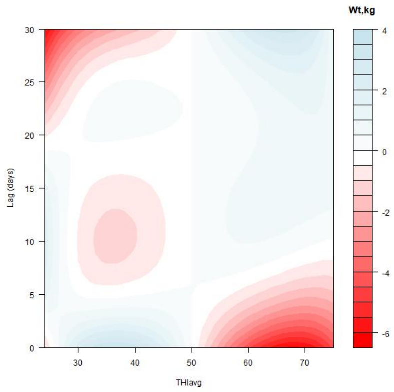
Figure 2 shows a one-dimension view of the expected changes in weight along the lag axis for three thermal load scenarios. The first scenario (cold) shows weight predictions along the lag axis for thermal loads below the 10th percentile of each variable. Similarly, the second (mild) and third (hot) scenarios represent changes in weight along the lag axis for the medium values and above the 90th percentile of each climatic variable, respectively. As previously pointed out for Figure 1, under the hot scenario, the thermal load in the closest days (0 to 5) to the day of recording predicted the largest negative change (loss) in weight, while under the cold scenario, thermal loads in the days further apart from the day of recording (25 to 30) were the ones with the highest negative effect on weight. Under the mild scenario, nearly no effect on weight was observed along the lag axis.

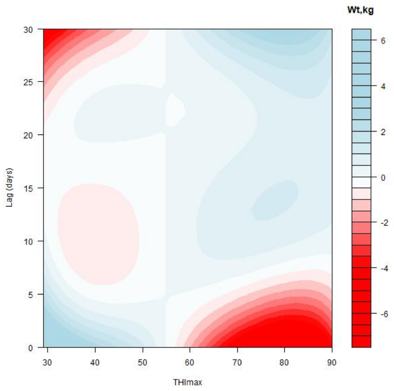
Both the maximum and average values of Ta or THI are those that predict larger weight losses for the warm scenario in the days close to the weighing day. For the cold scenario, the maximum and average values capture greater weight drops in the days close to 30 before weighing, while for the minimum values, the greatest weight drops are detected for lags 0.6

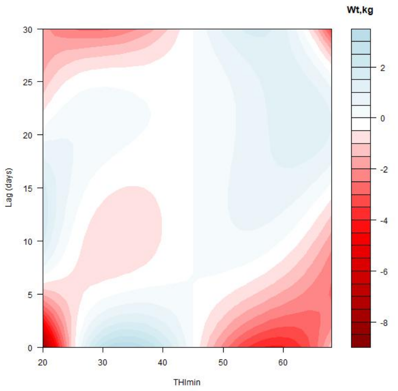

Wt,kg
5
0
-5
-10
Lag (days)
30
25
20
15
10
5
0
-5
0
5
10
15
20
25
30
Tavg (°C)

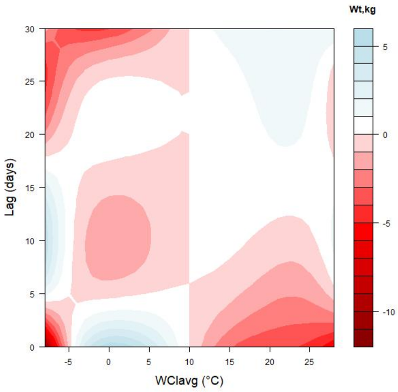

Wt,kg
4
2
0
-2
-4
-6
Lag (days)
30
25
20
15
10
5
0
0
10
20
30
Tmax (°C)

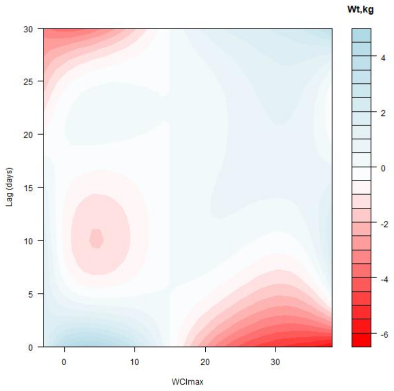

Wt,kg
5
0
-5
-10
-15
Lag (days)
30
25
20
15
10
5
0
-10
-5
0
5
10
15
20
Tmin (°C)

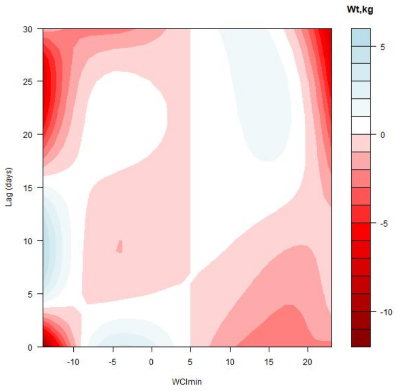

Wt,kg
4
2
0
-2
-4
-6
Lag (days)
30
25
20
15
10
5
0
30
40
50
60
70
THIavg

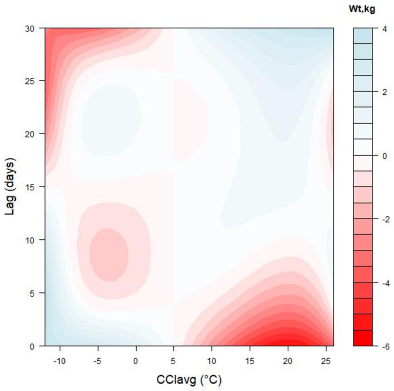

Wt,kg
6
4
2
0
-2
-4
-6
Lag (days)
30
25
20
15
10
5
0
30
40
50
60
70
80
90
THImax

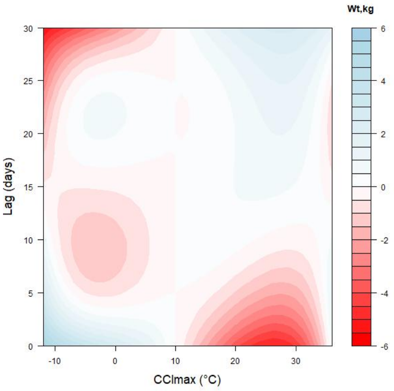

Wt,kg
2
0
-2
-4
-6
-8
Lag (days)
30
25
20
15
10
5
0
20
30
40
50
60
THImin


Wt,kg
5
0
-5
-10
Lag (days)
30
25
20
15
10
5
0
-5
0
5
10
15
20
25
WCIavg (°C)


Wt,kg
4
2
0
-2
-4
-6
Lag (days)
30
25
20
15
10
5
0
0
10
20
30
WCImax


Wt,kg
5
0
-5
-10
Lag (days)
30
25
20
15
10
5
0
-10
-5
0
5
10
15
20
WCImin


Wt,kg
4
2
0
-2
-4
-6
Lag (days)
30
25
20
15
10
5
0
-10
-5
0
5
10
15
20
25
CCIavg (°C)


Wt,kg
6
4
2
0
-2
-4
-6
Lag (days)
30
25
20
15
10
5
0
-10
0
10
20
30
CCImax (°C)

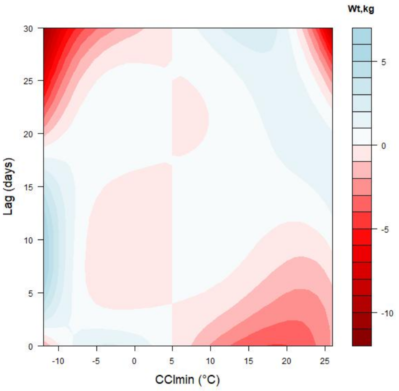
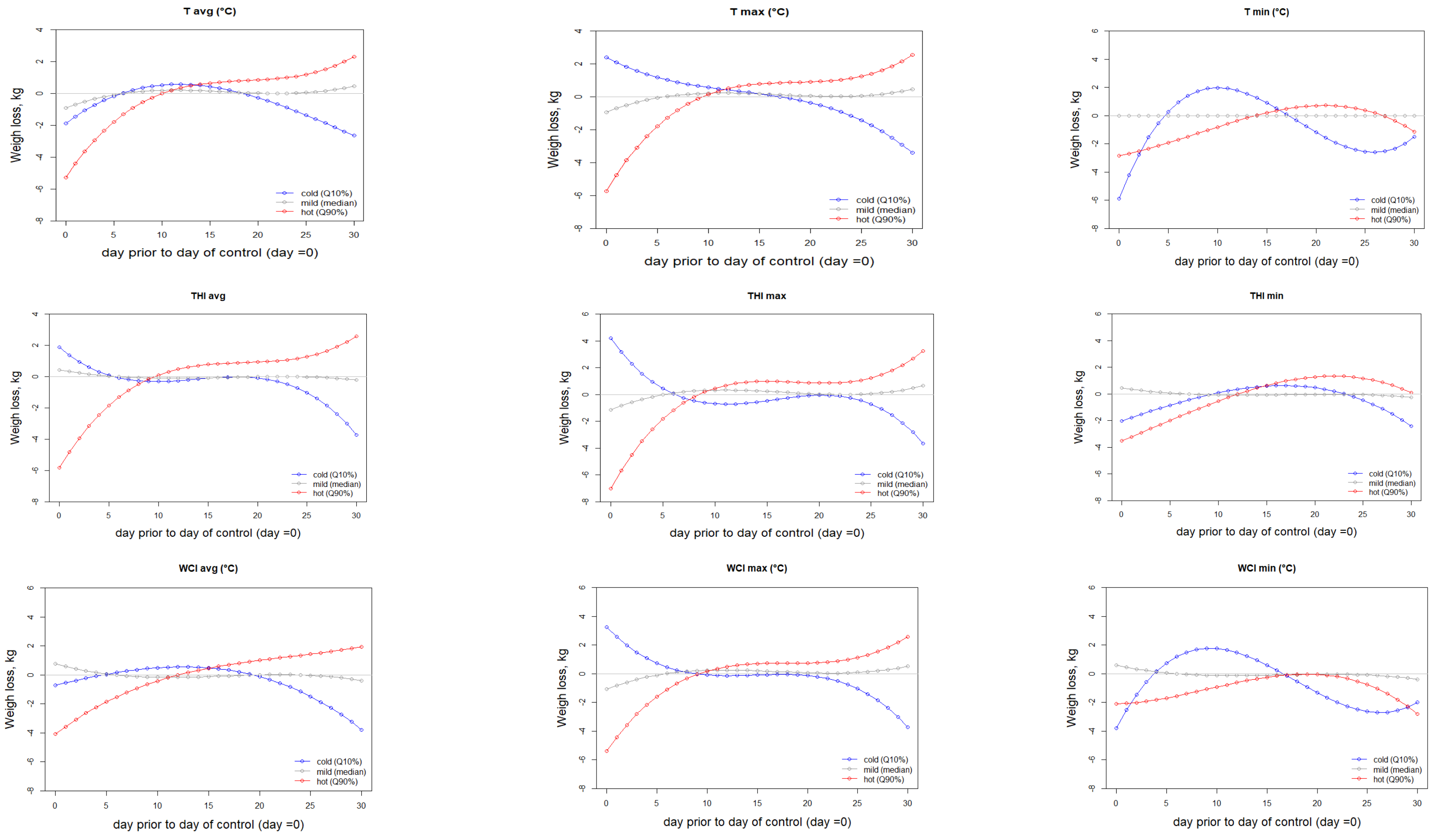

Wt,kg
5
0
-5
-10
Lag (days)
30
25
20
15
10
5
0
-10
-5
0
5
10
15
20
25
CCImin (°C)

Figure 1 Weather-weight relationship at lags between 0 and 30 days previous to recording. $^{1}T_{avg/max/min}$ =daily average/maximum/minimum temperature; $RH_{avg/max/min}$=daily average/maximum/minimum relative humidity; $THI_{avg/max/min}$ = daily average/maximum/minimum temperature – humidity index; $WS_{avg/max/min}$ =daily average/maximum/minimum wind speed; $WCI_{avg/max/min}$ = daily average/maximum/minimum wind chill index; $CCI_{avg/max/min}$ = daily average/maximum/minimum comprehensive climate index;


T avg (°C)
T max (°C)
T min (°C)
THI avg
THI max
THI min
WCI avg (°C)
WCI max (°C)
WCI min (°C)
Weigh loss, kg
day prior to day of control (day =0)
cold (Q10%)
mild (median)
hot (Q90%)
0
5
10
15
20
25
30
-8
-6
-4
-2
0
2
4
6

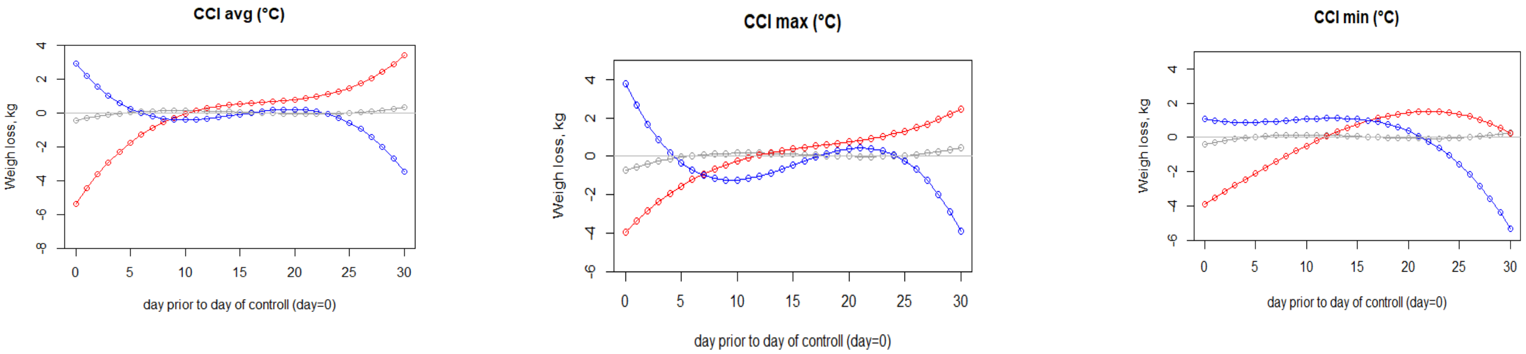


Figure 2 Lag effect of thermal load on weight from 30 day before control to the day of record (d 0) for overall data. Lines represent the weight losses estimates at 10th percentile (Cold (P10)), mild (median) and at 90th percentile (hot (P90)) of each climatic measure scale.

3.3 Assessment of Thermotolerance Thresholds

The study also aimed to characterize the production response to varying thermal loads through the estimation of the thresholds that define the thermal comfort zone (Figure 3). Results from the Gasparrini's algorithm in R (2011) have shown that the thermal load on the day of record (lag 0) provided a better fit than using thermal loads from previous days. Thus, the least square means for each climate variable at the day of recording, obtained from mixed model [2] were used in a piecewise-linear regression procedure to characterize the thermal thresholds and the slopes of production decay (Muggeo, 2003).Table 6 shows a summary of the estimated thresholds of decay due to thermal load for each climate variable. Model including the daily average temperature and WCI measures (Tavg, WCIavg) were those with a better fit of the weight data, with small differences within the models due to the high correlations between variables. As expected, a similar comfort zone was estimated for daily average Temperature and WCI, with a wide range of units [0.3, 25.5] °C for Tave and [1.47, 24.5] units for WCI. CCI avg showed the highest range of thermal zone, comprised between -6.42 and 23.7 units of CCI. This index has been designed to reflect the surroundings of the animal accumulating in a value the effects of Tave,, WSave and RHave.

Figure 4 shows the raw patterns and quadratic adjustment of the effect of all climatic descriptors on weight at feedlot. As expected, for comfort zone, slopes tended to be close to zero for all the climatic descriptors considered in this study. Thus, it indicates that weights at feedlot did not seem to be affected in the comfort region estimated between the threshold's values provided in Table 5. On the contrary, values of larger magnitude were observed outside the comfort region. The slopes decay of weight associated with thermal stress outside the estimated thresholds are summarized in Table 6. In the cold region, positive values correspond to decline in weight as we go below the cold threshold.

On the contrary, a negative value of slope is associated with a weight loss above the hot threshold, for the hot region. Except for THI, all climatic descriptors involving Tave, showed a larger impact on the performance of animals at feedlot. CI intervals for all slopes presented in Table 6 were different than zero value. Below the cold threshold, the greater decay was found for CCIavg. High positive values were also reported for WCIavg (2.20 kg), Tavg (2.16 kg), WCImin (2.13 kg) and CCImax (1.20 kg). Maximum and minimum daily temperatures, as well as average and maximum values of THI and maximum WCI did not capture large weight declines below the cold threshold (Table 6) and negative slopes were observed upper the hot threshold. For Heat stress, estimated slopes were -1.65 kg/Cº, -1.83 kg/per unit and -1.90 kg/per unit for Tavg, WCIavg, and WCImin, respectively where in all cases were values bellow the features estimated in the cold region. On average, the effect of stress of an ANI animal at the feedlot will be on average 1.9 kg per ºC

Table 5. Estimated thresholds the comfort zone for weight of each climatic variable.

Climatic variable	**Thr$_{cold}$^{1}**	**Thr$_{hot}$^{2}**
T_{avg}	0.3	25.5
T_{max}	5.6	27.69
T_{min}	7.03	18.39
THI_{avg}	50.7	67.7
THI_{max}	40.67	73.9
THI_{min}	31.53	53.62
WCI_{avg}	1.47	24.5
WCI_{max}	12.33	31.32
WCI_{min}	-8.52	17.65
CCI_{avg}	-6.42	23.7
CCI_{max}	- 3.73	11.8
CCI_{min}	-1.63	16.86

Thr$_{cold}$^{1}= cold threshold; Thr$_{hot}$^{1}= hot threshold; $T_{max/avg/min}$ =daily average,maximum, minimum temperature, respectively; THI $_{max/avg/min}$ =daily average,maximum, minimum temperature- humidity index, respectively; $WCI_{max/avg/min}$ =daily average, maximum, minimum WCI index, respectively; CCI $_{avg/max/min}$ = daily average/maximum/minimum comprehensive climate index;

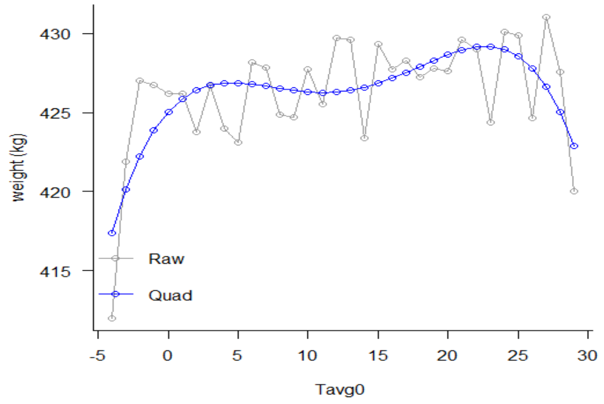
Table 6. Estimated slopes outside the comfort zone for each production trait and climate variable for the day before weighting (day = 0).

Climatic variable	Threshold	Estimated Slope	Std.err[1]	CI (95%) Lower[2]	CI (95%) Upper[3]
Tavg (°C)	Cold	2.16	0.25	1.65	2.67
	Comfort	0.13	0.01	0.10	0.16
	Hot	-1.65	0.25	-2.16	-1.14
Tmax (°C)	Cold	-0.18	0.02	-0.22	-0.15
	Comfort	0.14	0.00	0.13	0.15
	Hot	-0.05	0.01	-0.07	-0.03
Tmin (°C)	Cold	0.18	0.02	0.14	0.22
	Comfort	0.31	0.04	0.22	0.39
	Hot	-0.88	0.14	-1.17	-0.60
THIavg	Cold	0.06	0.01	0.04	0.08
	Comfort	0.19	0.01	0.16	0.22
	Hot	-0.44	0.04	-0.52	-0.35
THImax	Cold	-0.57	0.02	-0.61	-0.52
	Comfort	0.10	0.00	0.09	0.11
	Hot	-0.14	0.01	-0.17	-0.11
THImin	Cold	-1.19	0.05	-1.30	-1.09
	Comfort	0.20	0.01	0.18	0.23
	Hot	0.12	0.02	0.07	0.17
WCIavg (°C)	Cold	2.20	0.29	1.61	2.80
	Comfort	0.13	0.02	0.09	0.16
	Hot	-1.83	0.29	-2.42	-1.23
WCImax (°C)	Cold	0.00	0.00	-0.01	0.00
	Comfort	0.12	0.00	0.11	0.12
	Hot	-0.14	0.01	-0.16	-0.11
WCImin (°C)	Cold	2.13	0.60	0.90	3.35
	Comfort	0.30	0.02	0.25	0.34
	Hot	-1.90	0.38	-2.68	-1.13
CCIavg (°C)	Cold	4.49	0.46	3.56	5.42
	Comfort	0.14	0.03	0.08	0.20
	Hot	-2.59	0.65	-3.91	-1.28
CCImax (°C)	Cold	1.20	0.09	1.02	1.38
	Comfort	-0.01	0.03	-0.08	0.06
	Hot	0.15	0.02	0.12	0.18
CCImin (°C)	Cold	-1.22	0.07	-1.35	-1.08
	Comfort	0.37	0.02	0.32	0.42
	Hot	-0.70	0.10	-0.90	-0.51

[1] Std.err: Standard error;

[2] CI (95%) Lower: lower limit of the 95% Confidence Interval

[3] CI (95%) Upper: upper limito f the 95% Confidence Interval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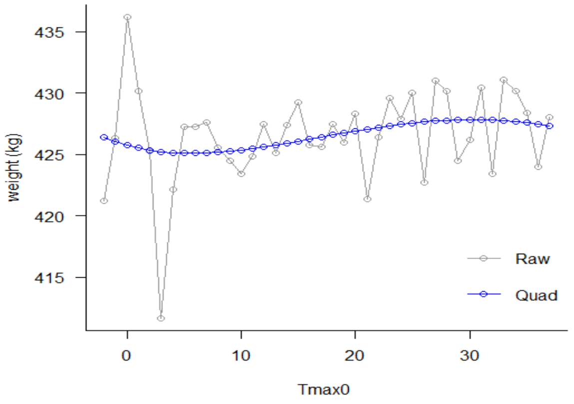
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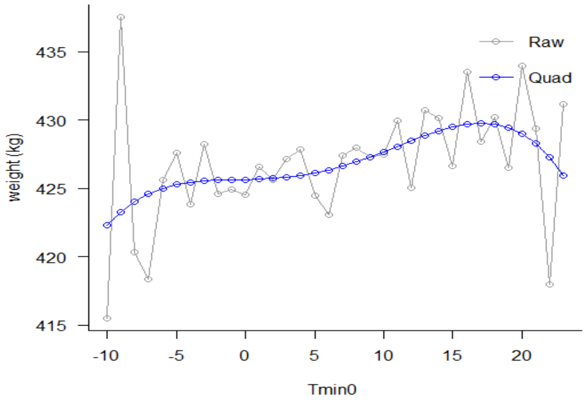
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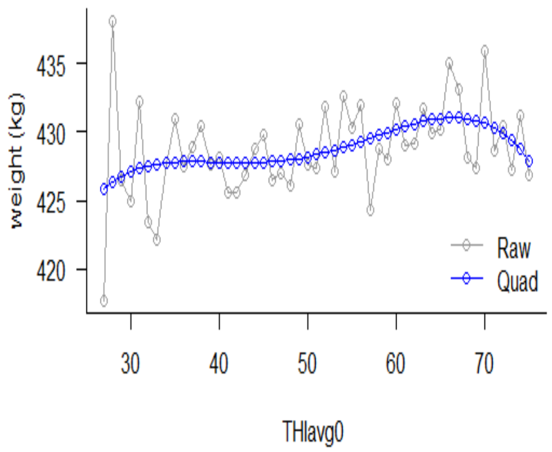
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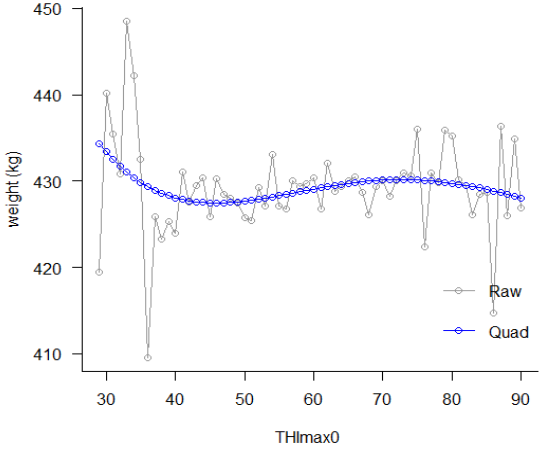
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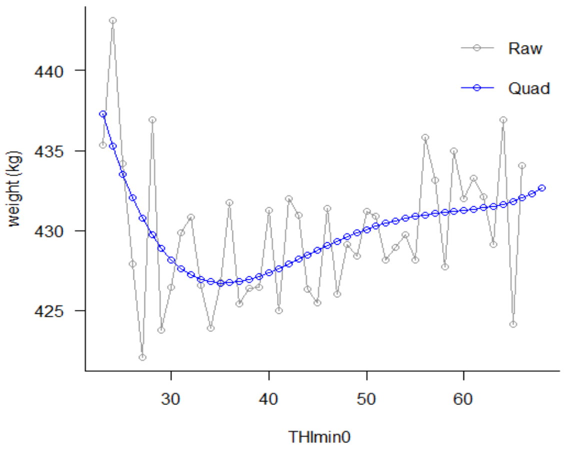
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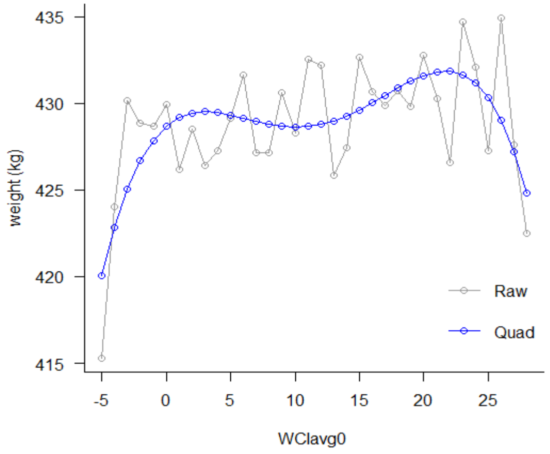
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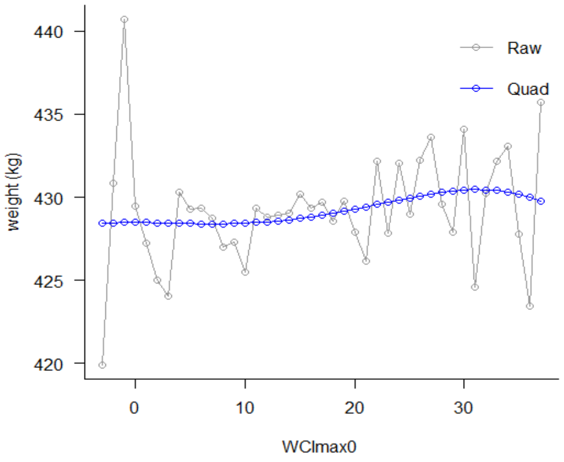
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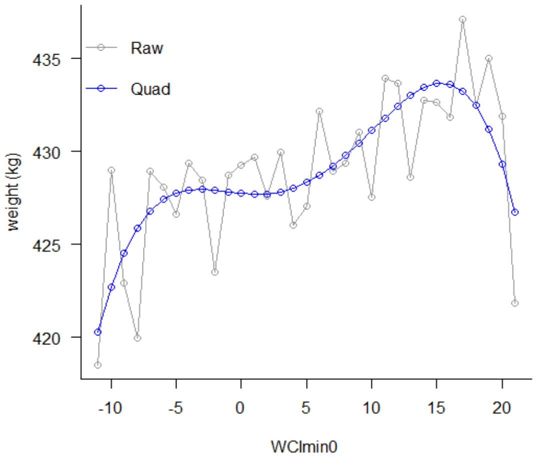
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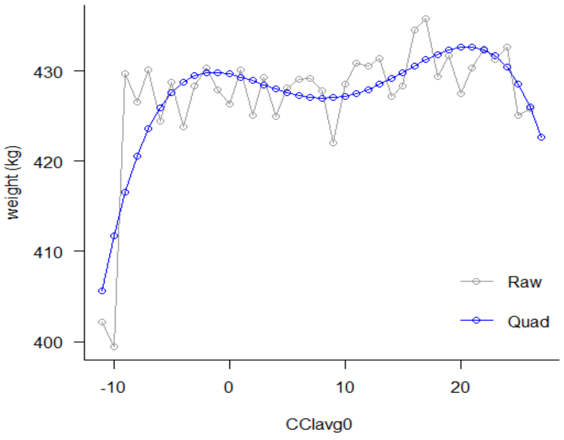
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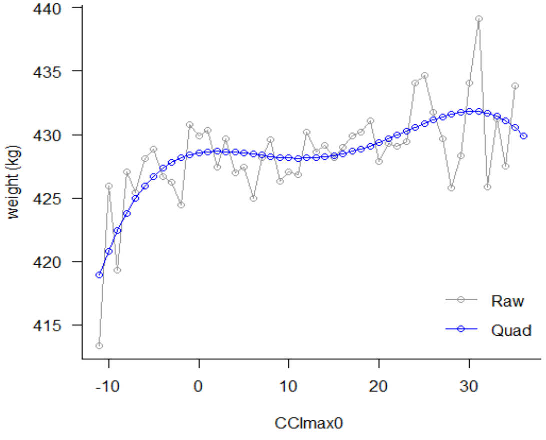
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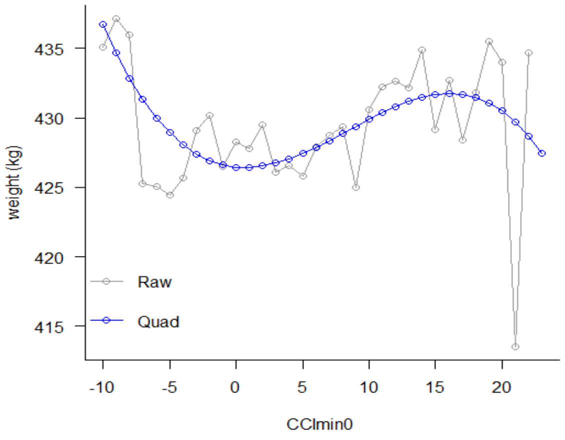
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Figure 4. Estimated effect of temperature and climatic indices measured in the day of recording on feedlot weight for each climate variable, trend for d 0 (day of controll). [1]$T_{avg/max/min}$ =daily average/maximum/minimum temperature; $RH_{avg/max/min}$=daily average/maximum/minimum relative humidity; $THI_{avg/max/min}$ = daily average/maximum/minimum temperature – humidity index; $WS_{avg/max/min}$ =daily average/maximum/minimum wind speed; $WCI_{avg/max/min}$ = daily average/maximum/minimum wind chill index; $CCI_{avg/max/min}$ = daily average/maximum/minimum comprehensive climate index;

4. Discussion

Research activity in the area of the effect of thermal stress on performance of livestock has been mainly focused on modeling the effect of heat stress on dairy traits in different species, such as dairy cattle (Ravagnolo et al., 2000, Hammami et al., 2013, Bernabucci et al., 2014, Carabaño et al., 2014) , dairy sheep (Finocchiaro et al., 2005; Peana et al., 2007a, b ; Ramón et al.,2016), dairy goats (Menendez-Buxadera et al., 2012). However, in beef cattle much work has been oriented to evaluate mitigation strategies for heat-stress at feedlots (see, Brown-Brandl, 2017) but little has been done to evaluate and model the effect of thermal stress on production traits (Santana et al., 2015; Bradford et al, 2016). Moreover, to our knowledge there is lack of comprehensive studies addressing the effect of thermal stress (cold and hot) on the performance of beef calves at feedlot. We have faced the present study as a previous step to deepen on the genetic bases of thermal response.

4.1 Estimation of Lag effect of Thermal Load on weight

To characterize the effect of thermal stress on performance, it is necessary to determine the effect of different climatic descriptors (loads) as well as to identify when the time of exposure to the stress (lag) better reflect the effect on the producing animal. In that respect, THIavg and lag between 0 and 3 days before the date of recording have been estimated in dairy cattle (Bernabucci et al., 2014) and dairy sheep (Finocchiaro et al 2005). On the contrary, for beef data, the accumulated effect of THI for a period of 30 days prior the recording of weaning weights and up to six months for yearling weight were assumed (Bradford et al., 2016) in order to estimate the genetic variability associated to the thermal stress response. Results reported in this study have also evidenced that climatic conditions affect ANI calves' performances at feedlot. In Figure 1, we show the effect of different thermal loads and lags on weights of animals at feedlot. In general, when there is a negative impact of climatic conditions, we could assess that those climatic conditions on the day of recording (lag 0), have the largest negative impact on the weight of ANI calves. This is particularly clear under heat stress (Figure 1) for Tavg. This is even more evident considering changes in weight predicted in a bi-dimensional space (the lag and the thermal load scale) in Figure 2. Under those scenarios, the model predicts the effect of a constant load during a certain lag (up to 30 days). Under the hot scenario, represented by loads above the 90th percentile of each climatic descriptor (red line), the largest negative effect on weight would occur during the closest days to the day of recording (days 0 and 5). This is evident for Tavg. Then, for all variables considered in this study the impact of heat load rapidly declined over the lag period (Figure 2) while there is a steady state previously to that lag revealing a period of adaptation. As many authors have pointed out, the effect of a continuous heat stress on weight it is a clear consequence of the need to maintain homeostasis (see Brown-Brandl 2017). The most evident adaptation response of animal to heat stress is the reduction in feed intake to diminish heat metabolic production (Mader et al., 2001; Brown-Brandl et al., 2005; Brown-Brandl et al., 2006; Valente et al., 2015). Moreover, if heat stress periods were long enough (heat-waves), animals would have an additional energy demand that will require a temporary increase of body reserves mobilization to maintain thermoregulation mechanisms such us panting, sweeting, increase heart rate (Baumgard, 2012; Gaughan et al., 2010) what in the end would acute the loss of weight.

From the perspective of cold stress scenario, the picture is more heterogeneous. While looking at Tavg the opposite trend was observed. suggesting that a stronger adaptability of this breed to cold conditions derive from a delayed impact of cold events on weight over the considered lag period (Figures 1). The adverse impact of cold scenario really depended on the range of values considered for each climatic variable (Figure 1). For average and minimum climatic variable, the cold side showed the negative effect on weight after a period of exposure of an average of 20 days. The weight drop occurred at the day before the weighting when the values were below -10°C for Tmin and -10 WCImin units and below 25 units of THImin. The same trend was observed also considering maximum values of CCI since the range of CCImax was wider than those reported for other maximum climatic variables: the coldest side of CCImax was below -10 units. However, in terms of lags, indices including RH under continental climate have an admixture impact, because in this type of climate high RHs are associated to low T^a (see, Table 3) what diminishes the apparent temperatures. Similarly, to the heat-stress scenario, changes in weight predicted in a bi-dimensional space (the lag and the thermal load scale are also showed at Figure 2. When cold stress, represented by thermal loads bellow or equal to the 10th percentile (line in blue) first starts (lag 30) there is a loss of weight that decreases while animal is accommodating its needs to the "cold stage". This is so till it gets to the point where that recovering status reverts to be again transformed in loss of weight. This is particularly evident when considering minimum (Tmin and WCImin) and average (Tavg, WCIave) loads. Trend illustrated for cold scenarios represents normal mechanism of adaptability to low temperatures from animals (Hoffman, 2010). Within a "thermoneutral zone", animals do not have to expend any extra energy to maintain their body temperature. When temperatures start to decrease, the animal must increase its metabolic rate to supply more body heat and combat the cold stress (Klain and Hannon, 1969). In a first phase, cattle may compensate cold stress with an increase of feed intake in an effort to meet their increased energy requirements. If subjected to a further cold period, the increased feed intake will not be enough to maintain the additional energy requirements, resulting in an ulterior loss of body mass that will be used to produce metabolic heat (Klain and Hannon, 1969). In this study, what we have observed is that exposure to cold event is prolonged over more than 20 days, the amounts of additional feed required for thermoregulation resulted in a negative energy balance and in a consequently loss of weight that will be more apparent around the recording date and of smaller magnitude to that of heat stress.

Characteristics of individual animals of this breed can also position them at higher risk for heat stress because of the color of its coat. In a study conduct on crossbreed steers, Mader et al., (2002) found that dark-colored cattle had the greatest percentage of cattle showing moderate to excessive panting score, while light-colored cattle displayed the least panting. Then, the better adaptability of these animals to the relatively low temperatures could be due to the greater susceptibility of European Bos taurus breeds to high temperatures compared to the breeds which evolved in tropical regions (Hahn., et al 1985).

4.2 Assessment of Thermotolerance Thresholds and effect of thermal stress on weight

For this breed, the adaptation to extreme climatic conditions has been established by the assessment of thermotolerance thresholds or the estimation of the thermo-neutral zone (Figure 4 and Table 5). We define the thermo-neutral zone as the range of environmental loads where animals do not need to spend additional energy to maintain homeostasis and therefore, they efficiently allocate feed supply to satisfy their production needs, in our case to increase weight. As expected, thresholds varied according to the climate descriptor (see Table 5) because each descriptor incorporates different sources of stress that change the apparent T^a (Mader et al, 2010). In terms of average T^a the comfort range estimated in ANI breed is longer than that refer for animals in Northwest feedlots in USA (Hristow et al., 2018) where calves start to experience the impact of heat stress below 0ºC and above 20 ºC. It is true that in those environmental conditions the apparent T^a as a result of wind speed and RH maybe different that the one experience by ANI calve. Moreover, breeds fattened in those feedlots are different from ANI and it is well known that differences among breeds on the physiological strategies to cope with harsh conditions exist (Ghaugan et al., 2010; Pereira et al, 2014).

The upper and lower thresholds of the different climatic variables appear to be enhanced by the effect of HR% and WS (Table 5). We could observe that the estimated lower critical threshold [0.3°C for Tavg] was in line with the lower value reported for CCIavg [-6.42 °C] which confirms that effects of Tavg are modulated by RH and WS in this breed/specie as well as in other species (Mader et al., 2010). The upper critical threshold, 25°C of average temperature (Tavg), was similar to those observed by Brown-Brandl et al. (2005) who reported a reduction of 17 % in feed intake for unshaded heifers when average ambient temperature increased from 19.7 °C (maximum 21 °C) to 27.7 °C (maximum 35 °C). The same author also reported a depression in feed intake of 3–5 % when ambient temperature increases from 25 to 35 °C with an intake reduction go beyond 30 % when temperatures exceed 35 °C.

Table 6 shows the estimated slopes outside the comfort regions for all climatic variables. There are two major finds. We have been able to estimate slopes of non negligible magnitude for Tavg, WCIave, WCImin and CCIavg for both cold and heat stress (bellow and above the thresholds mentioned before). According to our results, ANI calves under current conditions experience more loss of weight at cold stress than at heat stress. The estimated slopes are between 1.3 and 1.7 larger for cold than for heat stress for Tavg and CCIavg respectively. These results seem to be in contradiction with those previously reported in Figure 2. However, we have to take into account that Figure 2 shows the result of a continuous constant exposure (lag) to the heat of cold load however, what we show here is the impact of lag 0 at the current loads event occurring in this commercial feedlot during the period of study. The environmental conditions at this feedlot prevent animals to suffer from a prolonged heat stress. Even though during summers Tavg may go above the upper thresholds, the gradient of T^a during the day (Table 3) are large enough to provide opportunities to these calves to alleviate the heat stress. Apparently calves in feedlots require from 1 to 3.5 hours to recover from thermal stress particularly in hot periods (Mader et al., 2005). On the contrary, environmental conditions in this particular area during the cold period are more stable with low gradients of climatic variables (Table 3), therefore calves are exposed to longer periods of continuous cold stress. We have been using three different indices in addition to temperature. From the estimates of the correlation of climatic variables the Tavg is a climatic variable that show very high and the largest correlations (above 0.85) with the others. In that respect, maybe the use of Tave is has the easiest and more coherent interpretation.

ANI breed is a local breed considered to be adapted to their production system however, we have shown a relative impact of climatic variables on the performance of calves at feedlot. Nevertheless, little is known on what is the impact of growth potential on the sensitivity to beef animals to thermal stress. In this regard, it would be of great interest to evaluate and quantify the effect of climatic events on calves from a breed subject to a higher selection pressure. In addition, it is always argued that more stream breeds are always more sensitive to limited harsh conditions (Hanh et al., 1985; Gaughan et al, 2018) than local breeds. As a result, the choice of a breed for a given limiting environment has been proposed as a strategy to mitigate the impact of thermal stress on production of livestock species (Gaughan et al, 2018). However, from what we have seen here, with climate change evolving toward a more constant exposure of animals to heat stress, this type of studies should be done to monitor the effect of heat stress and determine if thermal response should be included in the selection of this breed while selecting to improve their genetic potential.

The genetic component of thermal tolerance for the Spanish local breed ANI, as well as results obtained from the investigated existence of GxE interactions across the wide thermal range has been reported in Chapter 4.

5. Conclusions

The present study aimed to address the effect of adverse climate conditions on performance of Avileña-Negra Ibérica (ANI) local beef cattle reared in the Castile - Leon area of Spain. This region is characterized by the presence of extreme temperatures during long periods throughout the year. The study of lagged effect of thermal load showed that conditions on the test day (lag 0) and the 2 d or 3d before mainly determine how ANI calves respond to thermal stress. The study of relationships between the weight trait and climatic variables allowed us to consider the average daily temperatures (Tavg) as an appropiate predictor and identify a wide comfort zone, between 0.3 and 25.5°C in which animals could expressed their highest production ability. Models predict that if ANI calves were subjected to constant temperatures (above threshold) the effect of heat stress would be twice as much as the one of cold stress. However, under the current climatic conditions where these animals are fattened, cold stress results in the largest loss of weight. Thus, the impact of environmental conditions on these calves' performance at feedlots will very much depend on how climate evolves in this area.

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Chapter 4: Individual response to thermal stress of Avileña Negra-Ibérical calves at feedlot

Abstract

The increasing demand for meat in developing countries, together with to the rapidly increasing rate of the world's climate conditions has made the thermal tolerance one of the most important adaptive aspects for cattle. The identification of thermally-tolerant breeds and also the selection of more resistant animals within the same breed is basic to maintain the productivity of the beef cattle production systems.

In this study the aim was to investigate the genetic component of thermal response in male calves of Avileña-Negra Ibérica (ANI) and to develop a genetic evaluation to improve thermal tolerance using reaction norm models in the context of RR. Two random regression models (RRM) with second and third order of Legendre polynomials for additive genetic and permanent environmental effects were fitted to estimate the reaction norm of these two components of weight at feedlot to climatic variables. A total of 29,591 weight records belonging to 5,876 animals collected between 2005 and 2017 were used. The pedigree file included 15, 418 animals. The RRM were fitted for weight using average daily temperature as covariable through use of Blupf90 programs. Average daily temperature at the location of the feedlot ranged from a minimum of - 4 to a maximum of 30 °C. Results obtained from M1 and M2 revealed a low genotype by environmental **(GxE)** interaction due to a strong adaptability of ANI to adverse and extensive environmental conditions.

EBV for changes in weight at different temperatures were estimated for all animals. Model 2 had the best fit to the data. The results revealed a mean of variation of the EBV values of 197.8 kilograms within each temperature class. The range of average variation between the minimum and maximum temperature class was equal to -114.5 and 83.69 kg, respectively. Additionally, among the temperature scale a range of the hereditability equal to 0.52 and 0.64 was recorded.

Keywords Thermal tolerance. genotype × environment interaction. Beef cattle. Weight. Reaction norm.

1. Introduction

European areas will encounter different effects of the changing climate and its impact on agriculture and livestock production will vary for the different geographical regions (Miraglia et al., 2009). These changes can substantially affect the cattle growth and reproduction performance with an important economic impact on livestock production (Hahn, 1998; St-Pierre et al., 2003). There is an ample evidence of the effects that chronic thermal stress could have on beef cattle productivity (McManus et al., 2009) what in many developing countries has been seen as an increase in the intensification of animal industries and a shift to cross-breeding animals that have higher levels of productivity than many indigenous breeds (Hoffmann and Beate, 2006).

The genetic component of thermal tolerance has been studied in several species such as dairy cattle (Ravagnolo and Misztal, 2002; Carabaño et al., 2018), pigs (Fragomeni et al, 2015), beef cattle (Santana et al., 2015; Bradford et al., 2016) and dairy sheep (Finocchiaro 2005). In dairy cattle there is a strong genetic antagonism between level of production and milk production (Carabaño et al., 2016). However, adaptation to high temperature environments and high production potential seems to be very limited in beef cattle (Santana et al, 2015; Bradford et al 2016). In this regard, selection strategies to improve level of production without losing resilience is more plausible.

Spain, as part of the Iberian Peninsula, is considered a hot spot in the Mediterranean basin regarding climate change (Segnalini et al., 2011). Thus, it has and will have to face the effect of climate change on its livestock sector. In Spain, beef production is to a large extent based on local beef breeds and their crosses. The Avileña Negra Ibérica (ANI) is one of the most relevant ones with an expansion in the area of more influence of the heat waves, characterized by a continental climate of short cold winters and very dry and warm summers. ANI cow-calf production is done in extensive and harsh conditions with low producing pasture during large periods of the year. The post-weaning phase is in feedlots to avoid cows and calves having to compete for the limited feeding resources. In the previous chapter, we have determined the effect of thermal stress on weight of calves at feedlot. ANI calves appeared to be sensitive to thermal stress. Heat loads at recording time have an acute effect on weight however during summer time, this effect is diminished during the day because these animals are under a wide gradient of temperature that somehow mitigates the acute effect of heat. On the other hand, during winter time such mitigation process does not occur, therefore the cumulative effect of cold stress has a larger impact on weight. Thus, if we want to establish sensible selection strategies that help producers to search for more productive animals without damaging their plasticity to environmental changes, it is necessary to know if there are individual differences in the responses to thermal stress.

The genetic component of thermal tolerance has been studied in several species such as dairy cattle (Ravagnolo and Misztal, 2002; Bernabuchi et al, 2014; Carabaño et al., 2016), pigs (Fragomeni et al, 2015), beef cattle (Santana et al., 2015; Bradford et al., 2015) and dairy sheep (Finocchiaro et al. 2005). In dairy cattle, there is a strong genetic antagonism between level of production and heat tolerance and signs of GxE interaction between dairy production under cold, comfort and hot periods (Carabaño et al., 2016). Much less information exists in beef cattle for which extrapolation to different breeds and production systems are also more cumbersome than for dairy production.

The purpose of this study was to investigate the genetic component of thermal response in male calves of the Spanish local breed ANI, as well as to explore the existence of GxE interactions across the wide thermal range endured by these animals. The results are expected to provide useful information to define breeding strategies to improve growth potential without losing adaptation to harsh weather conditions.

2. Materials and Methods

2.1 *Animals and data*

Data were provided by the Avileña-Negra Ibérica breed association (Avila, Spain) and comprised 29,591 weight records from purebred animals of the Avileña Negra-Ibérica beef cattle breed. Records were collected from 5,876 animals and dated from September 2005 through October 2017. Pedigree file included 15, 418 animals.

Weights were obtained in the commercial feedlot of Riocabado, a municipality located in the region of Castile and Leon, Spain, with a mean of 5 records (SD = 1.21) for each animal (Table 1). Details about the rearing system of the animals included in this study have been provided in Chapter 3.

Based on the analysis conducted in our previous work, in order to model the individual response of calves to thermal stress we have used daily average temperature (**Tavg**) on the day of control as the thermal load and lag respectively. We have considered **Tavg** the most representative of weather conditions of the area under study and the variable better adapted to fit the thermal response of these animals.

Meteorological data were made available by the Spanish Agency for Meteorology (AEMET) from 3 different weather stations, Avila, Gotarrendura and Revilla de Barajas, all of them close to the feedlot location during the period from 1995 and 2017. Gotarrendura provided most of the meteorological information because of being closest to the feedlot. Only when missing information in the Gotarrendura station was found with respect to dates of weighing in the feedlot, information from the other two stations was used, resulting in a dataset of 8,283 records. Then, weight records were merged with the hourly average air temperature, for the corresponding period of study. The general trend of Tavg is descripted in detail in Chapter 3.

2.2 *Thermal Stress Analysis*

Two random regression models (**RRMs**) with second (**M1**) and third (**M2**) order of Legendre polynomials for additive genetic and permanent environmental effects were fitted to estimate the reaction norm of these two components on weight in feedlot to climatic variables.

The general model equation was:

$$y_{ijkl\_T} = CG_i + \sum_{p_j=0}^{3} \beta_{p_j} Z_{\beta p}(d) + \sum_{q=0}^{n} b_q Z_{bq}(T) + \sum_{s=0}^{n} a_{sk} Z_{as}(T) + \sum_{s=0}^{n} p_{sk} Z_{ps}(T) + e_{ijkl\_T}$$

[1]

where, y_{ijkl_T} is the observation for a given weight trait at a certain temperature value (Tavg) = T; CG_i is the contemporary group, defined as the combination of season and year of entry at the feedlot (i=1,…, 47); β_{p_j} are the regression coefficients defining the expected weight of animals at a given age d for class j of initial age at entering in the feedlot (j=1,…,4; 1= 130-195; 2=196-225; 3=226-258; 4=259-330 days) and $Z_{\beta pj}(d)$ are the matrices of covariates of the cubic Legendre polynomials evaluated at age d; b_q and $Z_{bq}(T)$ are the regression coefficients and matrices of covariates of Legendre polynomials evaluated at temperature T, respectively; a_{sk} and p_{sk} are the additive genetic (**AG**) and permanent environmental (**PE**) random regression coefficients for animal k, respectively, and $Z_{a/ps}(T)$ and are the matrices of covariates of the Legendre polynomials evaluated at T, and, e_{ijkl_T} is the residual effect, with e_{ijkl_T} N(0, σ_e^2). Quadratic (n=2 in equation [1], model M1) and cubic (n=3 in equation [1], model M2) Legendre polynomials were alternatively used to model the genetic and permanent environmental components.

The Legendre polynomial terms were calculated with the following recursive formula:

$$P_{n+1}(x) = \frac{1}{n+1}\left((2n+1) * x * P_n(x) - n * P_{n-1}(x)\right)$$

, where x is the standardized value of temperature T (Tavg in our case) in the [-1, 1] scale

$$x = 2\left(\frac{T - T\min}{T\max - T\min}\right) - 1$$

, and n refers to the nth order Legendre polynomial, with $P_0(x) = 1$ and $P_1 = (x) = x$.

The variance structure for the random regression coefficients for the additive genetic (**a**) and permanent environmental (**p**) components was assumed to be:

$$\text{var}(\mathbf{a}) = \mathbf{G}_o \otimes \mathbf{A}\ ;\ \text{var}(\mathbf{p}) = \mathbf{P}_o \otimes \mathbf{I}$$

, with $\mathbf{G}_o$/ $\mathbf{P}_o$ = (co)variances between random regression coefficients for the AG/PE component, **A** = additive genetic relationships matrix and **I** = identity matrix.

From estimates of the random regression coefficients for animal i, $\mathbf{a}_i$, the AG deviation value for weight at thermal load T, g_{iT}, was obtained from the expression:

$$g_{iT} = \mathbf{z}'(x)\mathbf{a}_i$$

, **z**(x) is the vector of Legendre polynomial covariables associated with each regression coefficient evaluated at standardised value x of Tavg=T

Additive genetic and PE (co) variances for performance under thermal loads T and T' were calculated as

$$\sigma_{g_{T,T'}} = \mathbf{z}'(x)\mathbf{G}_o\mathbf{z}(x') \; ; \; \sigma_{p_{T,T'}} = \mathbf{z}'(x)\mathbf{P}_o\mathbf{z}(x')$$

Heritabilities of weights at a given temperature (h_T^2) were obtained as

$$h_T^2 = \frac{\sigma_{g_T}^2}{(\sigma_{g_T}^2 + \sigma_{p_T}^2 + \sigma_e^2)}$$

Genetic correlations between weights taken under two temperatures, T and T' were also estimated from the $\sigma_{g_{T,T'}}$components:

$$\rho_{g_{T,T'}} = \frac{\sigma_{g_{T,T'}}}{\sqrt[2]{\sigma_{g_T}^2 \sigma_{g_{T'}}^2}}$$

Finally, slopes of the genetic deviation pattern of individual i at each value T (Slp_{iT}^{g}) were obtained from derivatives of the quadratic Legendre polynomial fitted to each individual as:

$$Slp_{iT}^{g} = \mathbf{c}(x)'\mathbf{a}_i$$

, with $\mathbf{c}(x)'$ containing the coefficients of the derivative of the quadratic ($\mathbf{c}(x)' = [0 \quad 1 \quad 3x]$) and cubic($\mathbf{c}(x)' = [0 \quad -1/2 \quad 3x \quad -15/2x^2]$) Legendre polynomial. Backtransformation of these slopes to the original scale of temperatures was obtained by multiplying by the factor range(x)/(range(T)=2/range(T).

The (co)variance between slopes at T and T' values of the thermal load was obtained as,

$$Gslp(T,T') = \mathbf{c}(x)'\mathbf{G}_o\mathbf{c}\,(x)$$

,and, the covariance between the level of production not dependent on the thermal load (intercept of the random regression) and the slopes at a given value T:

$$\text{Gint\_slp}=\text{cov}(\mathbf{k}'\mathbf{a}_i,\mathbf{c}(x)'\mathbf{a}_i)=\mathbf{k}'\mathbf{G}_o\mathbf{c}$$

, with **k**'= (1 0 0) being the vector that gets only the intercept from the vector of solutions of the random regression coefficients.

Solutions for each position and dispersion parameter involved in the model were estimated by using the gibbs2f90 program (Misztal et al., 2002).

Changes in weight at different temperatures were plotted by average temperature classes for 10 animals with the highest and lowest EBV.

3. Results and discussion

This study compared two alternative models, quadratic (M1) and a cubic (M2) random regression, to estimate the individual response in weights to changes in thermal loads. The cubic model provides a better fit of the analyzed data. The fact that a wide range of thermal loads is present in the data might lead us to expect the need for functions that allow for several changes in the trajectory to describe reaction to cold, comfort and hot temperatures. Nevertheless, estimates of the parameters of interest under both models ought to be evaluated before favoring one model over the other.

Table 3 shows estimates of (co)variances between additive genetic and permanent environmental random regression coefficients for quadratic (M1) and cubic (M2) models. These matrices were used to subsequently obtain genetic parameters of weight along the thermal load scale and for level (intercept regression coefficient) and slopes of change in weight along the thermal scale following formulae described previously. Estimates for additive genetic variances for level of the trait (a0), were larger than estimates for permanent environmental variance linked to individuals. Estimates are in accordance with previous values obtained for variance components of feedlot weights in this population.

Figure 1 shows estimates of additive genetic and permanent environmental variances along the Tavg scale under models M1 and M2. Results show a different trend between the AG and PE variances for different temperature conditions. Overall, similar estimates of the two variance components were observed under the quadratic and cubic models except for the extreme temperatures, with the cubic model showing smaller estimates than the quadratic model at high temperatures for the AG component and at cold temperatures for the PE effect. Estimated variances for the AG component showed a more stable pattern along the temperature range than PE estimates, which decreased substantially under hot conditions. In particular, using M1, the lowest AG was found in correspondence of the middle range of Tavg (between 5 and 25 °C) whereas the largest AG was estimated for extreme values of Tavg (under 5°C and above 26°C). The trend of AG reported for M2 decreased until a Tavg of 15°C to increase until 28°C. As regards PE, both models predicted similar variances for the higher Tavg range, but as temperature decreased, particularly under 5°C, M2 estimated lower PE than M1.

Heritability estimates (shown in Figure 2) were moderately high, ranging from 0.53 to 0.65, which are larger than other estimates of heritability of weight of calves reported by some authors (Bradford et al., 2016; AAA, 2016) but in line with those observed in the literature for Angus cattle (Meyer, 1999; Rumph et al., 2002; Costa et al., 2011). Lowest values of heritability were observed between 0 and 5ºC of average daily temperature and highest under warm conditions. The cubic model showed a slight decrease in heritability at the hottest temperatures. Thus, selection response for increase weight may be expected to be larger when selection decisions are based on weights measured under comfort or warm conditions than when weights are obtained under extreme weather. Zumbach et al. (2008) and Fragomeni et al. (2016) observed that the heritability for carcass weight in a commercial swine population was higher under heat stress. On the other hand, Cardoso and Templeman (2012), Santana et al. (2015) and Bradford et al. (2016) observed that heritability of weight in beef cattle was greater under more favorable environments.

Figure 3 shows estimated correlations between weights taken under different thermal loads for both models. Models M1 and M2 yielded similar correlations for weights taken at close temperatures but differed in the prediction of correlations of the estimated genetic value of weights taken under hot vs. cold temperatures. Under model M2, estimates of genetic correlations were above 0.8 (the commonly considered as threshold value for GxE interactions) for all combinations of temperatures. On the other hand, for the quadratic model, the lowest correlation value was 0.58 for weights measured under the hottest (Tavg=30 ºC) vs. the coldest (Tavg=-5ºC) thermal loads. Estimates of correlations for PE effects along the temperature scale are shown in Figure 4, showing similar or more patent discrepancies between models M1 and M2 than those observed for AG values. The well-known problems of fit at the extremes of the trajectory of polynomial functions might explain these discrepancies, especially in the case of estimates of covariances, for which estimation errors are normally higher than for variances. Overall, in this population, GxE seems to be small or moderate regarding weight achieved under a wide range of temperatures. Lack of GxE interaction for weights taken at different temperatures has also been reported by Santana et al. (2013). On the other hand, Bradford et al., (2016) found that estimated genetic correlations for the direct component of weaning weight were strong for modest heat load differences but decreased to less than 0.50 for large differences in Angus cattle. The larger growth rate of selected Angus cattle compared with local breeds might explain the higher levels of GxE found for this breed since intensive production might make animals more vulnerable to the environmental conditions. In fact, a stronger GxE interaction has been observed for dairy cattle across the range of THI (Ravagnolo and Misztal, 2002; Sánchez et al., 2009). The large difference of genetic correlations between beef and dairy cattle could be the results of different in their genetic potential for milk production that make dairy cattle more sensible to higher thermal conditions. Tolerance to elevated temperatures has declined in dairy cattle, and highly selected breeds of pigs and poultry because of the increasing metabolic heat production related to a high milk production and rapid growth (Zumbach et al., 2008; Dikmen and Hansen, 2009). On the other hand, the small GxE interaction found in our analysis is probably related with the adaptability of ANI breed to adverse environments. These animals are characterized by a high adaptability to arid conditions and by a strong capacity to travel long distances in search of pasture (Fernández-Perea et al. 2004). Thus, the adaptive advantages to extreme temperature conditions of these animals could results in a lower GxE interaction for the AG component of weight. Different authors have also stated that unselected locally adapted breeds are more resistant to warm environmental conditions (Berman, 2011; Gourdine et al. 2017; Santana et al. 2015). Hoffman et al. (2010) reported that most local breeds are well characterized by a better adaptation to extreme thermal conditions.

The selection criteria proposed to improve thermal tolerance would be the slopes of decay of weight under the more extreme conditions. Table 4 shows estimated correlations between the intercept, which represents the level of genetic potential for each individual for weight, and the slopes of the individual curves of response in weight to changing heat loads as well as the estimated correlations between slopes. Estimates of the genetic correlation between level of weight potential and thermal tolerance were very low or close to 0 (with the largest absolute value of the estimated correlations of -0.039 and -0.153 obtained for correlations between intercept and slope at -4ºC for models M1 and M2, respectively). These small values align well with the lack of GxE interaction showed in Figure 3 and indicate that selection to improve weight is not expected to have a large impact on thermal tolerance, at least under the current environmental and productive levels.

To further quantify the importance of GxE interaction, ranking of animals for EBV across classes of Tavg was investigated by drawing the individual response curves to temperature for the 10 bulls with the highest and lowest EBVs (Figure 5). Although animals remain within the best or worst group along the range of thermal loads, reranking of animals is observed within groups when EBVs for weight taken under cold vs high temperatures are compared, showing differences in thermal tolerance among individuals. In both groups, tolerant (animals that showing positive slopes along the temperature scale) and susceptible (those showing negative slopes) can be observed.

M2 had the best fit to the data. Bulls included in the higher group had a greater slope of production in line with the genetic antagonism between the production level and thermal resistance of animals (Ravagnolo and Misztal (2000); Santana et al., 2015). As shows in Figure 4, bulls under most stressful conditions would be expected to have a greater than 10 kg weight difference between low T class and maximum T class. On the contrary, for both models the lower group of animals seems to be more constant over the different T classes, revealing a greater resistance to extreme environmental conditions.

The weight differences reported in this study are smaller than those observed in Angus cattle by Bradford et al., (2016) who found a drop of weight between comfort and maximum heat load to be greater than 20 kg. It should also be noted that the slope under maximum T classes illustrated in Figure 5 could be underestimated due to the relative short duration of the hot season in this area and the expectedly high adaptability of this breed to extreme environmental conditions. As a consequence, few records had high values of Tavg. However, ANI breed is reared under a continental weather conditions, characterized by cold temperatures produced by incursions of polar air. Higher parts of Ávila’s region regularly suffer from frosts also during the summer season. Thus, these animals are exposed to air temperatures below critical temperature throughout the larger part of the period conferring to these animals the ability to regulate body temperature in response to cold stress and a greater sensibility to higher temperatures, in line with those observed in our previous study.

Taking into account the adverse environmental context where these animals are reared, selection to improve growth should not neglect adaptive traits such as thermal tolerance. The best option would be to select for individuals that rank highly in stressful and comfort conditions. Vercoe and Frisch (1992) stated that physiological requirement and high resistance are not mutual exclusive and the cross-breeding would be a good tool to maximizing the production under adverse environmental condition. As already proposed by Bradford et al., (2016), in order to implement the selection programs of this breed, the geographic location information of animals should be provided to combine heat tolerance for all of these traits into an index the breed associations and provide producers with comprehensive, easy-to-use selection tools.

4. Conclusions

Two random regression models (RRMs) with second and third order Legendre polynomials for additive genetic and permanent environmental effects were fitted to estimate the reaction norm these two components of weight at feedlot to climatic variables in males of Avileña-Negra Ibérica (ANI).

We found a large genetic correlation (>0.8) between weight merits at different T classes that revealed a low Genotype by Environmental interaction. Furthermore, 20 bulls from the validation population were selected as the most and less resistant individuals based on the greater and lowest estimated breeding value (EBV). Level of weight merit (Highest vs. Lowest EBV) does not change with T class. However, some re-ranking of animals is observed across T within each group.

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Table 1. Summary statistics for weights and age of calves at entry and exit of the feedlot and distribution of data according to the number of weights available per animal

Data distribution			Descriptive statistics			
N^{w1}	*Nr^{1}*	*%*	Stage	Parameter	Age (days)	Weight (Kg)
1	5876	100	*At entry in*	Mean	228	261
2	5876	100	*The feedlot*	Std^2	43	56
3	5876	100		Min	130	130
4	5400	92		Max	330	380
5	3751	64				
6	1828	31	*At exit of*	Mean	437	528
7	704	12	*The feedlot*	Std	51	35
8	187	3		Min	312	355
9	23	0		Max	620	600

[1] ***N^w***: number of weights per animal over the period of study. ***Nr*** *number of animals*

[2] Std: Standard deviation

Table 2. Summary statistics for weights and age of calves at entry of the feedlot and distribution of data according to the number of weights available per animal.

Age class of entry	Weight data (kg)				
(Days)	Max	Mean	Min	Std2	N^w
1(130 – 195)	374	221	130	41	1506
2 (195 – 225)	373	247	130	46	1446
3 (225 – 258)	380	272	136	50	1461
4 (258 – 330)	380	306	159	49	1463

[1] ***N^w***: number of weights per animal over the period of study

[2] **Std:** Standard deviation

Table 3. Estimated (co)variances between random regression coefficients (a_i, i=0,…,n) for the additive genetic (above diagonal or top in diagonal) and permanent environmental (below diagonal or bottom in diagonal) components under quadratic (M1, n=2) and cubic (M2, n=3) polynomial models.

M1	a0	a1	a2
a0	1098.10 616.68	-9.64	4.72
a1	-130.02	305.02 182.15	17.82
a2	-32.04	-2.88	14.95 4.09

M2	a0	a1	a2	a3
a0	1127.40 601.37	-11.46	16.84	-37.65
a1	-108.23	172.68 94.56	7.90	-93.49
a2	-27.19	-14.69	27.52 7.37	-1.77
a3	68.12	-75.20	11.44	52.73 64.03

Table 4. Estimated correlations between intercept (Int) and slopes of the individual response curves at values of average daily temperature (slp_Tavg) obtained from the covariance matrices of random regression coefficients for the additive genetic (above diagonal) and permanent environmental (below diagonal) components under quadratic (M1) and cubic (M2) polynomial models.

M1	Int	slp_-4	slp_1	slp_6	slp_11	slp_16	slp_21	slp_26	slp_30
Int	1.000	-0.039	-0.034	-0.028	-0.020	-0.012	-0.005	0.002	0.006
slp_-4	-0.089	1.000	0.983	0.927	0.835	0.718	0.596	0.482	0.400
slp_1	-0.172	0.994	1.000	0.980	0.921	0.833	0.733	0.634	0.561
slp_6	-0.260	0.975	0.993	1.000	0.980	0.926	0.853	0.775	0.714
slp_11	-0.352	0.939	0.970	0.992	1.000	0.983	0.940	0.885	0.839
slp_16	-0.441	0.886	0.930	0.967	0.991	1.000	0.987	0.956	0.925
slp_21	-0.523	0.817	0.874	0.925	0.965	0.991	1.000	0.991	0.974
slp_26	-0.594	0.736	0.804	0.868	0.924	0.966	0.992	1.000	0.996
slp_30	-0.641	0.666	0.742	0.815	0.882	0.936	0.974	0.995	1.000

M2	Int	slp_-4	slp_1	slp_6	slp_11	slp_16	slp_21	slp_26	slp_30
Int	1.000	-0.153	-0.141	-0.107	-0.016	0.021	-0.051	-0.091	-0.108
slp_-4	0.434	1.000	0.997	0.987	0.989	0.852	0.812	0.850	0.875
slp_1	0.468	0.999	1.000	0.996	0.992	0.816	0.770	0.812	0.839
slp_6	0.530	0.994	0.997	1.000	0.991	0.770	0.716	0.760	0.791
slp_11	0.555	0.977	0.980	0.986	1.000	0.836	0.781	0.817	0.842
slp_16	0.300	0.943	0.934	0.922	0.952	1.000	0.993	0.993	0.991
slp_21	0.258	0.959	0.949	0.933	0.946	0.994	1.000	0.997	0.992
slp_26	0.273	0.972	0.963	0.947	0.951	0.988	0.998	1.000	0.999
slp_30	0.284	0.977	0.969	0.953	0.954	0.985	0.997	1.000	1.000

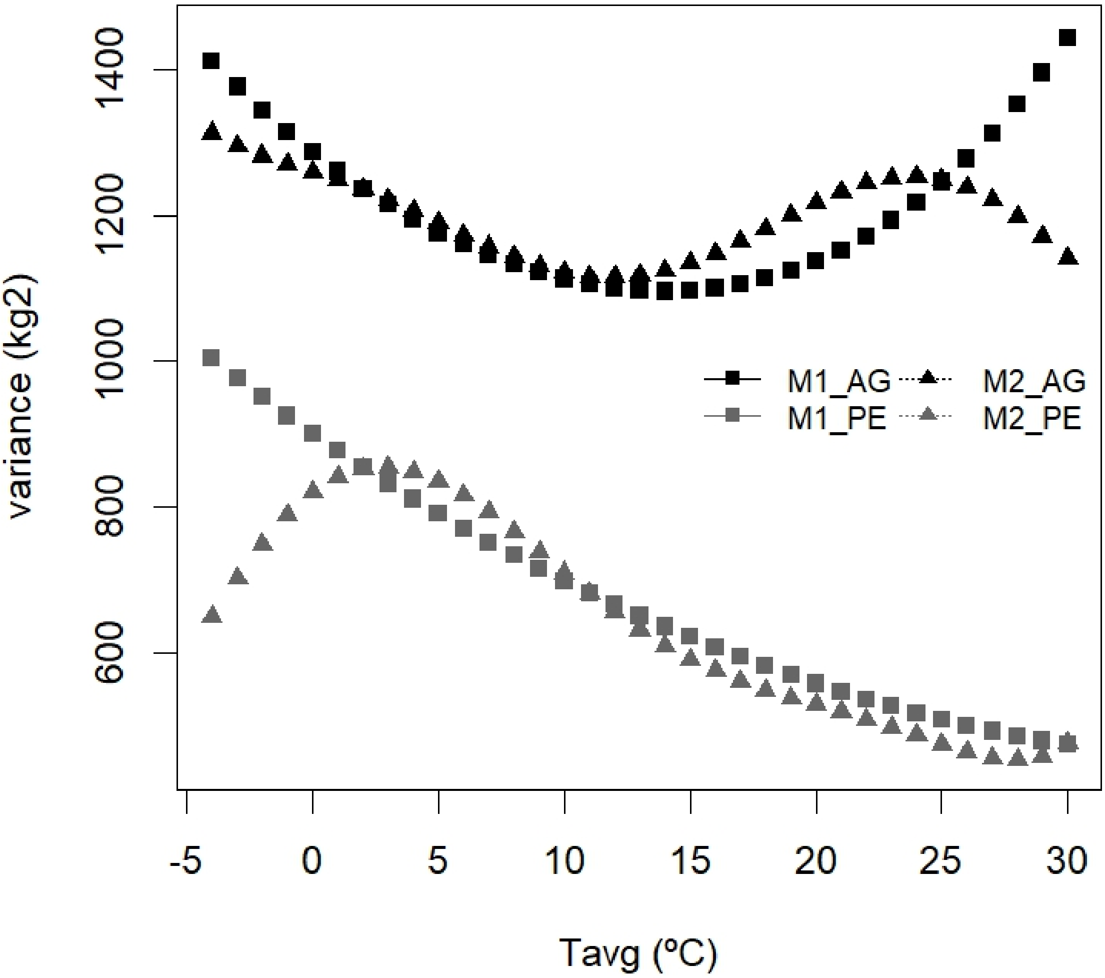

Figure 1. Estimates for additive genetic (AG) and phenotypic (PE) variance over the range of daily average temperature (Tavg) obtained under the quadratic (M1) and cubic (M2) polynomial random regression models.

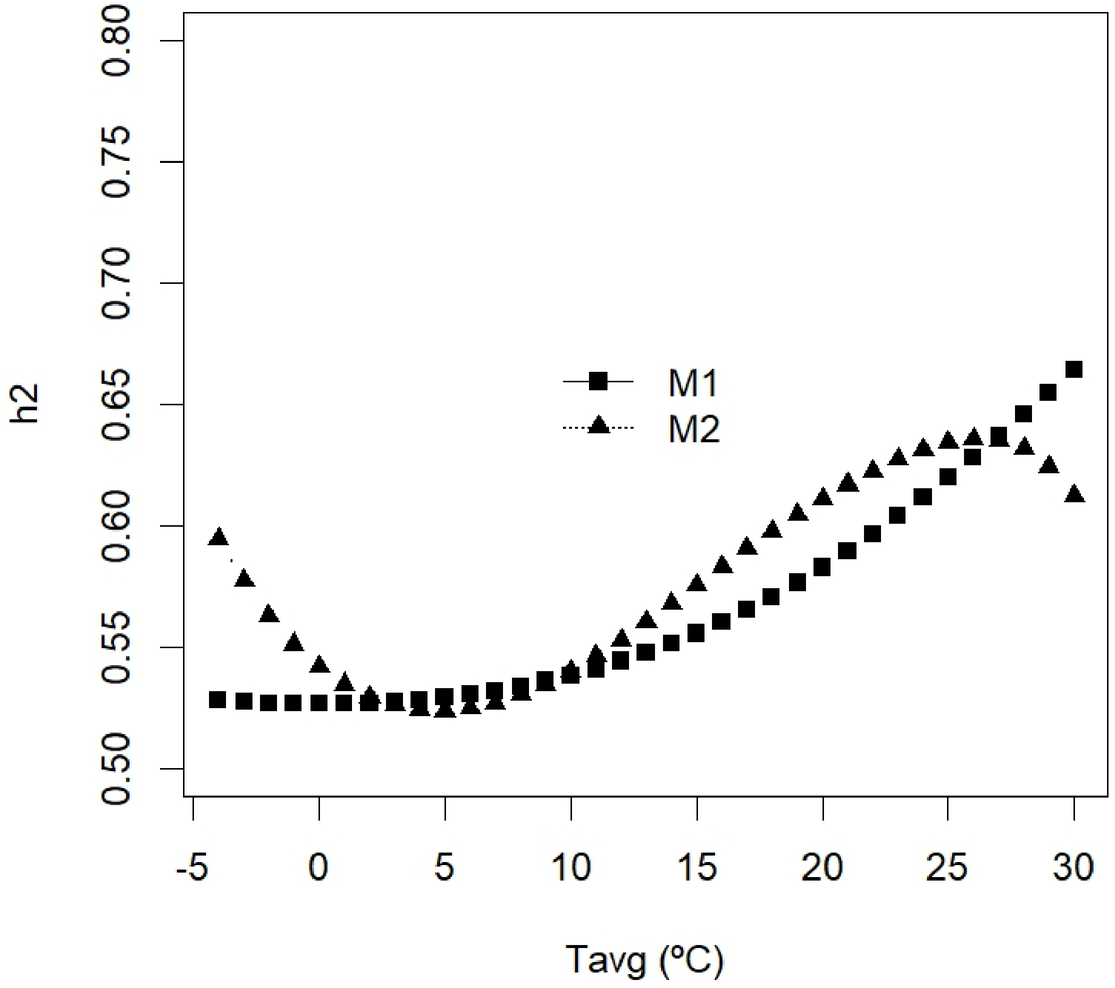

Figure 2. Heritability estimates for weight for different average temperature degrees (Tavg) obtained under the quadratic (M1) and cubic (M2) polynomial random regression models.

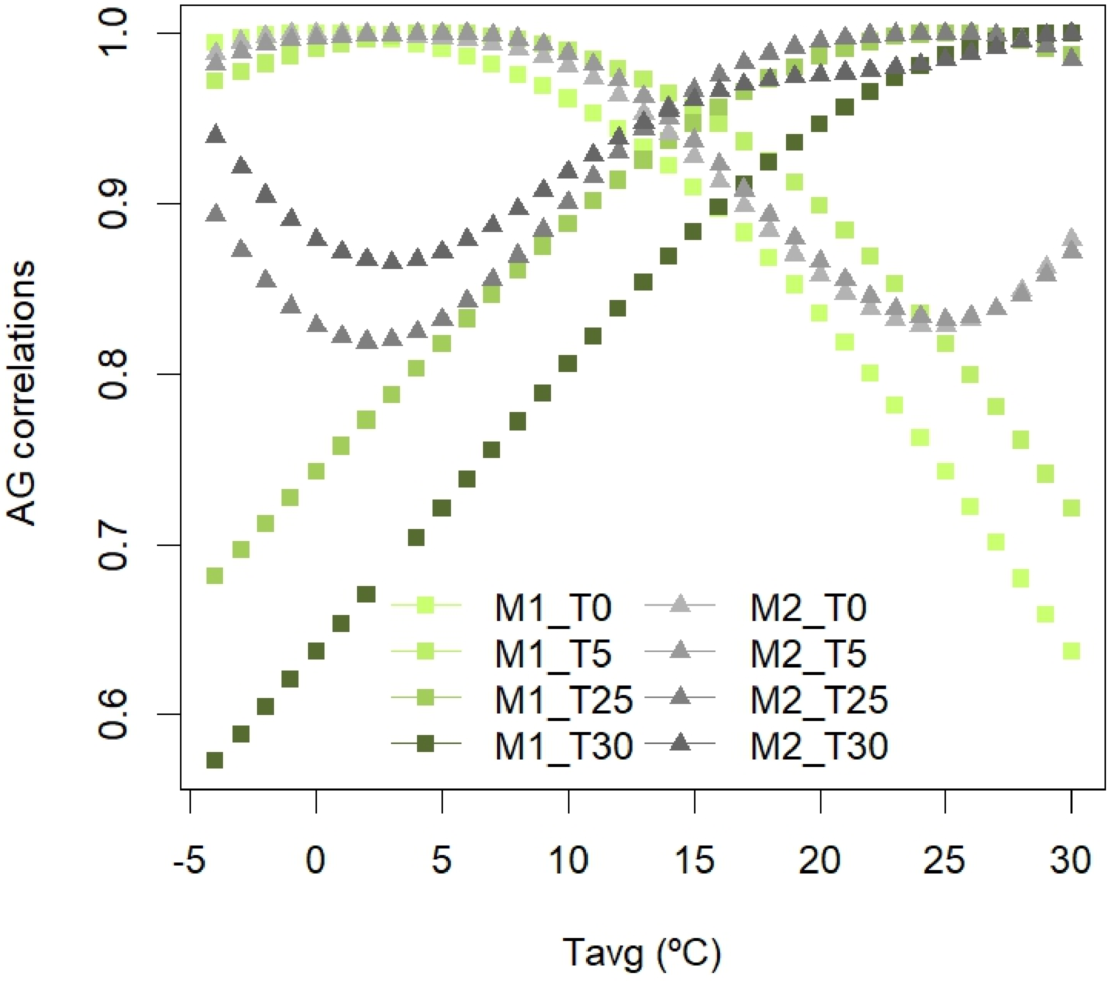

Figure 3. Estimated correlations between the additive genetic (AG) component of weight at 0, 5, 25 and 30 ºC of average daily temperatures (Tavg) with the AG components of weight along the whole range of Tavg values obtained under the quadratic (M1) and cubic (M2) polynomial random regression models.

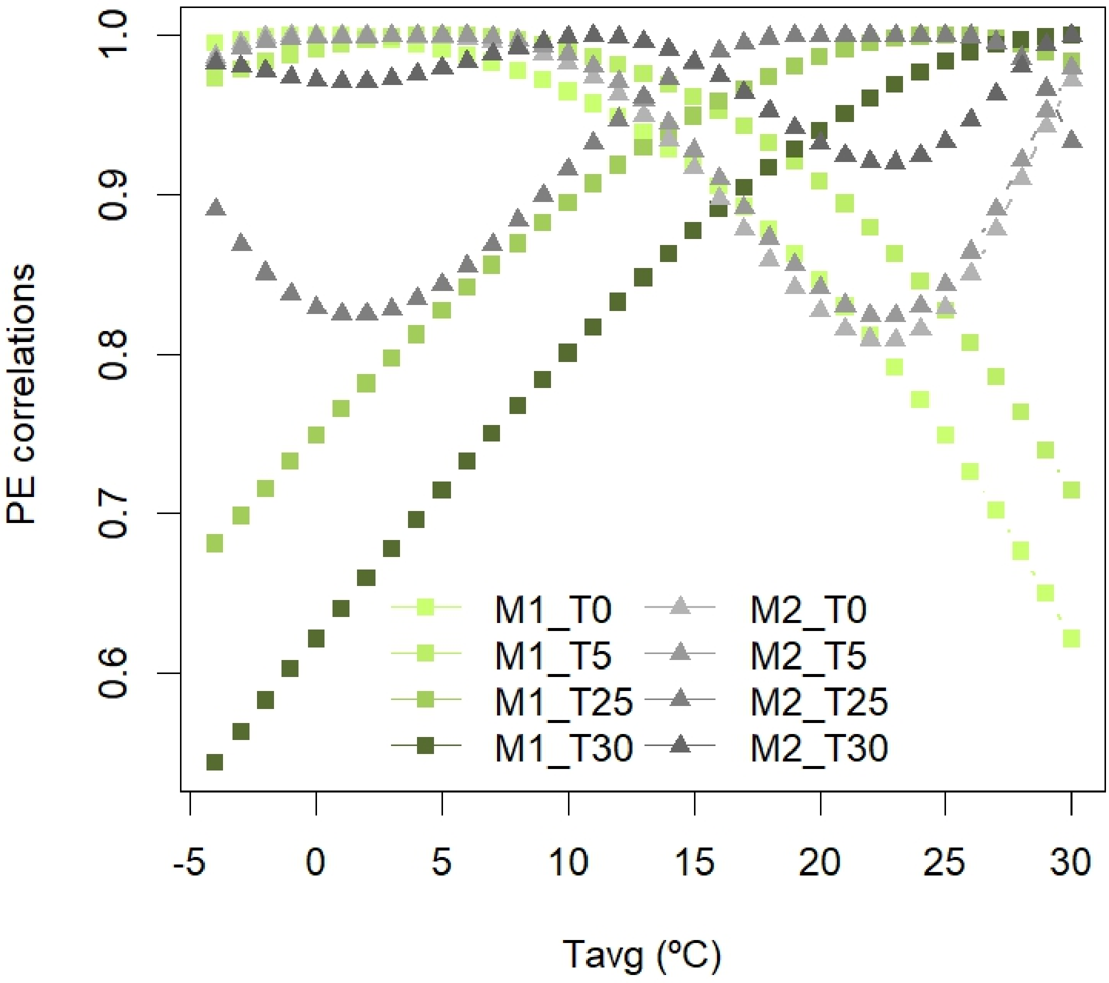

Figure 4. Estimated correlations between the permanent environmental (PE) component of weight at 0, 5, 25 and 30 ºC of average daily temperatures (Tavg) with the PE components of weight along the whole range of Tavg values, obtained under the quadratic (M1) and cubic (M2) polynomial random regression models.

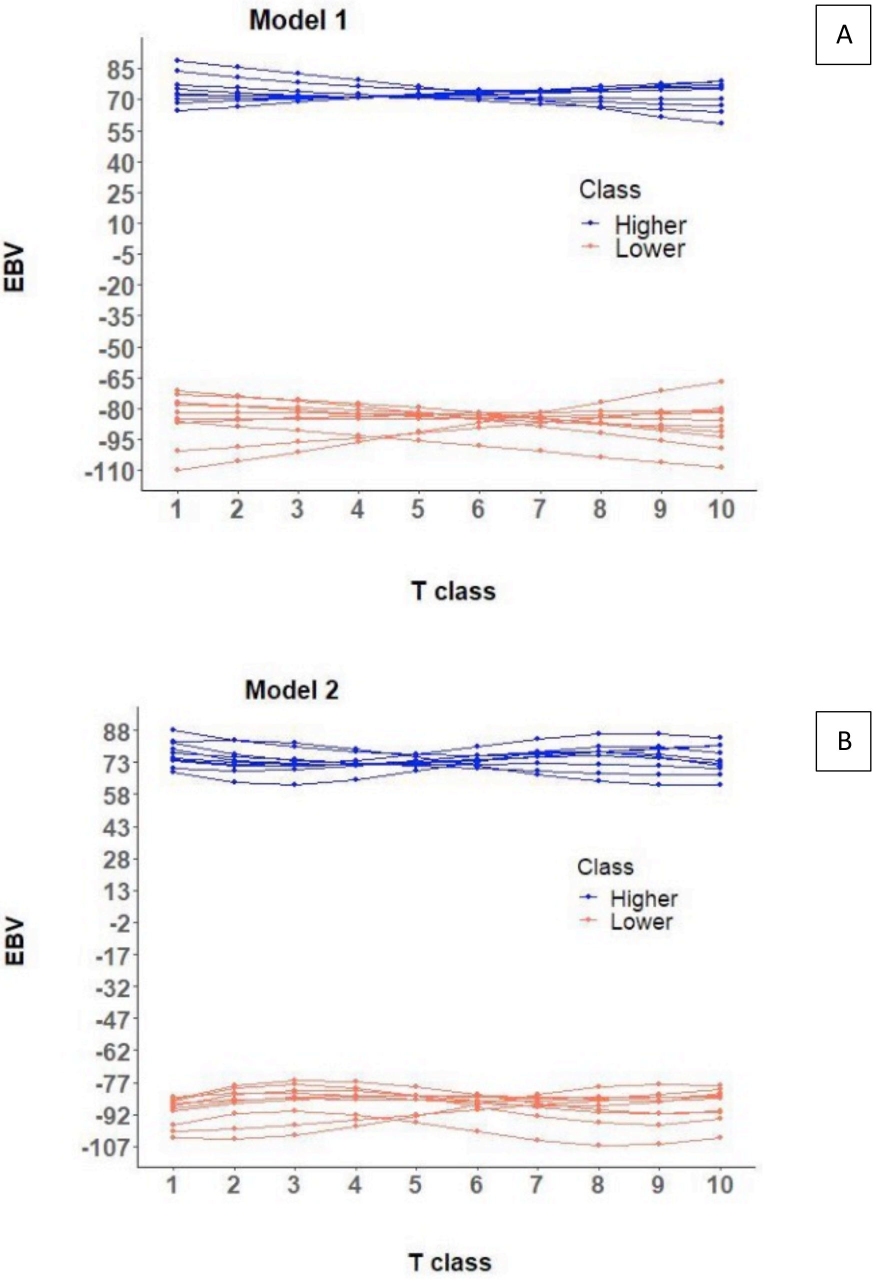

Figure 5. Changes of estimated breeding values (for weight by Average Temperature classes (T class) for the 10 animals with the Highest and Lowest EBV.

Chapter 5: Random Regression Model for The Evaluation of Heat Stress in Swine Carcass Quality Traits

Abstract

Genetic basis for carcass quality and characteristics associated with heat stress are of relevant importance because of their economic value in the pork industry. The aim of this study was to investigate the existence of a genomic - environmental interaction (G × E) for carcass quality traits in a reference population of crossbreed pigs. Data on 126,051 terminal crossbred pigs were combined with hourly values of air temperature (T, °C) and relative humidity (RH, %) recorded during the period between 2015 and 2019. The impact of heat stress was assessed using two different statistical models. A first linear regression (Model 1) model was fitted in order to investigate the response of backfat (BF), loin depth (LD) and average daily gain (ADG), to climatic factor ($RH_{avg, max, min}$; $T_{avg, max, min}$; $THI_{avg, max, min}$). In a second step, a genomic random regression model (gRRM) was implemented considering the best combination between the climatic predictor and thermal load period obtained from each run of Model 1. Based on solutions obtained from gRRM, the estimated breeding values (EBV) were calculated based on the higher and lower thermal resistance to rank the sires. Among the analyzed traits, BF was the most heritable with low-medium value ($h^2 = \sim 0.32$) corresponding to lower (63%) and higher (82%) range of relative humidity. On the other hand, ADG has been reported as a very low heritable trait ($h^2 = 0.16$) for low temperatures comprised between -7°C and 4°C. Conversely to the high values obtained for BF and HA (range between 0,75 and 0.99), the weakest genetic correlation (less than 0.50) was found for LD and ADG at different values of Tavg. Overall, results obtained from this study showed evidence of different genetics underlying phenotypes in different temperature and humidity, suggesting the existence of genotype × environment interaction.

Key-word: carcass quality traits, pigs, heat stress, genotype by environment interaction, random regression model.

1. Introduction

The increased relevance of heat stress to United States livestock industries is due to the estimated economic losses from heat stress on livestock production system across the United States. The animal economic loss derived from heat stress range between $299 to $316 million. Of these losses, those for grow-finish pigs are estimated to be $202 million (St-Pierre et al., 2003).

The biological mechanism by which heat stress impacts production and reproduction in pigs has been widely documented by different authors (Pearce at al., 2013; Fernandez et al., 2015; Johnson et al., 2015). However, very little is known about the detrimental effects of heat stress on carcass and meat quality traits in pigs.

Carcass characteristics, jointed with meat quality and growth rate represent an optimum combination for consumer satisfaction and consequently for a better competitive ability of pork on the market. Therefore, the knowledge of the genotype by environment interactions and the ability of pigs to perform during environmental heat stress conditions are required for US swine populations to implement selection programs and increase product quality for carcass traits.

Currently, selection tools for improving heat tolerance or adaptability are not routine in US national swine evaluations. Many studies explored the genetic variation in heat tolerance in several livestock species (Ravagnolo et al., 2000; Zumbach et al., 2008a,b; Carabaño et al.,2016; Tiezzi et al., 2017) using reaction norm models, suggesting the existence of a genetic determinism of heat tolerance. However, most of the studies regarding the G×E interaction occurred in swine population have focused their attention on live body weight and growth performance (Merks, 1989; Bidanel and Ducos, 1996; Godinho, R. M., et al., 2018, 2019). The same method was used by Zumbach et al.

(2008b) to evaluate the carcass weight heat tolerance in purebred and crossbred swine populations.

The original developments proposed by Ravagnolo et al. (2000) provided the basis of most of the studies conducted to implement genetic evaluations for heat tolerance on different species all over the word (Bradford et al., 2016; Fragomeni et al., 2016b; Bohlouli et al., 2019). The use of random regression models allows modeling the effect of a genotype as a function of environmental conditions through the estimation of genetic parameters over the range of an environment-dependent covariate (Legarra et al., 2009; Santana et al., 2016).

The objective of this study were: i) to estimate genetic parameters for heat tolerance using a random regression model coupled with genomic information in order to assess a potential genotype × environment interaction on carcass quality traits of commercial crossbred pigs; ii) to determine the usefulness of the genomic information identifying heat-tolerant individuals based on the higher and lower thermal resistance.

2. Materials and Methods

2.1 Animal data

Animal use approval was not needed for this study because the data were from an existent data base and were provided by The Maschhoffs, LLC (Carlyle, IL, USA). Loin depth (**LD**), backfat (**BF**) and carcass average daily gain (**ADG**), were measured on 126,051 terminal three-way crossbred pigs. The animals were a progeny of Duroc sires and different dam lines. Also, the age at the day of harvest (**HA**) was considered in order to better understand the dynamics behind the variation in ADG.

Animals phenotyped were raised during the period September 2015 to November 2019 on 2 commercial flows. The initial data set was composed by 135,768 records which has been edited in order to remove possible outliers. In particular, the data of each trait that were outside the mean plus or minus three times of the standard deviation were consider outliers and removed before any statistical analysis. Thus, flows 1 provided 37,400 records, while flows 2 contributed 88,651 records.

All the animals were divided in 57 different groups (**CG**) based on the combination of farm–month-year of birth ranged from 153 to 6,025 individuals for group. Table 1 shows the descriptive statistics of the final data set.

Piglets were moved to different nursing/finishing facilities after a weaning age of 18.7 ± 4.11 days. Individuals were considered ready for harvest when the approaching the weight of 136 kg, which occurred at 178 d ± 10.6 d of age. The distribution of the individuals based on month of birth and harvest record is shown in Table 2.

During the grow-finish period a typical standard pelleted diet were used based on sex and live weight of the animals. Individuals were monitored daily and received standard vaccination and emergency medication. For details on diet composition, vaccination, and medication during nursery, growth and finish periods see Lu et al.

(2018). Harvest dates were recorded to assign a slaughter batch. Thus, in this study the batch group was created take into account the concatenation of farm - month - year of harvest date (n = 84). Carcass quality traits were measured 24 h postmortem using a Fat-O-Meter system (Frontmatec A/S, Kolding, DK). The pedigree file animals traced back of 9 generations, including a total of 2,248 animals. Phenotyped individuals were progeny of 407 sires, 279 of them were genotyped using the Illumina porcineSNP60 BeadChip (Illumina, Inc., San Diego, CA, USA). Sires showed from 1 to 546 litters and sired from 1 to 18 crossbred individuals.

2.2 Weather data

Meteorological data consisted of hourly values of air temperature (**T, °C**) and relative humidity (**RH, %**) measured during the period between 2015 and 2019. As temperature/humidity data recorded inside the facility were not available, records were extracted from 3 different weather stations*, Springfield, Quincy and Lawrenceville,* distributed in the state of Illinois (IL, US). Weather stations were assigned to farms based on the zip code. Then, the nearest station was selected as the most representative of the environmental-climatic conditions, due to the closest proximity to the reference farm.

Based on hourly data we calculated average daily values of each climatic variable. Moreover, the temperature-humidity index (**THI**) was calculated considering that temperature and humidity influence much of the heat exchange impacts of warm and hot thermal environments. Hourly temperature-humidity index was calculated using the formula proposed by Zumbach et al., (2008b) as follows:

$$\mathrm{THI} = \mathrm{T} - (0.55 - 0.0055 * \mathrm{RH}) * (\mathrm{T} - 14.5)$$

where T was the observed temperature in degrees Celsius (°C), RH was the observed relative humidity on a 0 to 100 scale. Hourly values were then used to calculate daily average THI (**THI$_{avg}$**).

2.3 Statistical analysis

2.3.1 Quantification of heat stress

In order to better investigate the patterns of thermal stress, three lifetime periods were defined. Using the birth date of the animal, thermal load was defined for three-time intervals (60 to 92; 92 to 122 and 122 to 152 days) which resulted in start and end dates for each period for each individual in the study. Average daily Ta, RH and THI for each time interval was calculated. The nine environmental covariates (three time periods by three climate variables) where then merged to each individual's phenotype. A linear regression was fitted in order to evaluate the response of a considered trait to a certain environmental covariate (RH; Ta; THI).

Combinations between the environmental covariate and each carcass quality trait resulted in 36 different models which accounted for the cumulative effects of heat stress for the referred period.

The environmental covariates (as combination of climatic predictors and the thermal load interval) for each analyzed trait were selected considering the highest coefficient of determination (R^2) obtained from the following model:

$$y_{ijklmn} = \mu + CF_i + Par_j + G_k + \sum_{o=1}^{q} \mathbf{Z}_o(w) + \varepsilon_{ijklm} \quad [1]$$

where y_{ijklm} = is one of the four traits (BF; LD; ADG; HA). The fixed effects were CF = cross-fostering, i=1-2; Par = parity of the dam, j= 1-8; G = gender for the individual, k = 1-2. $\mathbf{Z}_o(\mathrm{w})$ are the covariates of the oth Legendre polynomial evaluated at climatic variable w; where o are 1st, and 2nd order Legendre polynomials for their respective climatic factor; ε_{ijklm} is the residual.

The polynomial Legendre included in model [1] were calculated with the followed formula:

$$\emptyset_{n\,(w)} = \sqrt{\frac{2n+1}{2}}\ P_n(w)$$

$$P_{n+1}(w) = \frac{1}{n+1}\left((2n+1)\ w\ Pn\ (w) - nPn - 1(w)\ \right)$$

where *n* is the *th* order of polynomial Legendre, $P_0(w)$ =1 and P_1 = (w)=w.

Then, each climatic record was standardized at the same interval by:

$$w = 2\left(\frac{w_i - w_{min}}{w_{max} - w_{min}}\right) - 1$$

where w is the mean of standardized climatic variable. w_i, w_{min} and w_{max} are the row mean values of each no-standardized minimum and maximum climatic values, respectively. The goodness of fit measured by coefficients of determination ($\mathbf{R^2}$) for each climatic factor and thermal load period is represented in Table 3.

Model analysis

In a second step, only the best combination between the climatic predictor and thermal load period obtained from each run of model [1] was used to estimate the effect of heat stress on each carcass quality trait. The other variables were excluded to reduce the complexity of the study. Genomic random regression model (**gRRM**) was implemented using the MCMC package of R (Hadfield, 2010) according to the following formula:

$$\mathrm{y_{ijklmn}} = \mathrm{CF_i} + \mathrm{Par_j} + \mathrm{G_k} + \sum\nolimits_{\mathrm{o=0}}^{\mathrm{q}} \mathrm{S_o}\mathbf{Z}_{\mathrm{o}}(\mathrm{w}) + \sum\nolimits_{\mathrm{o=0}}^{\mathrm{q}} \alpha_{\mathrm{lo}}\mathbf{Z}_{\mathrm{o}}(\mathrm{w}) + b_{\mathrm{m}} + \mathrm{l_n} + \varepsilon_{\mathrm{ijklmno}} \quad [2]$$

where, $y_{ijklmno}$ is the phenotype (BF, LD, ADG or HA); CF is the fixed effect of cross-fostering, i =1-2; Par is the fixed effect of parity, j= 1-8; *G* is the fixed effect of gender, k= 1-2; S_o is the o$^{\mathrm{th}}$ fixed regression coefficient specific for each class of climatic variable; α_{lo} are the random regression coefficients for additive genetic effect of sire l; $Z_o(w)$ was the vector of covariates of the o$^{\mathrm{th}}$ Legendre polynomial evaluated at each climatic variable; *b* is the random additive genetic effect of batch, m = 1-84; *l* is the random additive genetic effect of Litter, n = 1-20,252; ε_{ijklmn} is the random error $[N(0,\sigma_e^2)]$.

Genomic random regression models were implemented with the first-order polynomial. The (co)variance structure for model [2] was assumed to be:

$$\mathrm{Var} = \begin{bmatrix} \alpha \\ e \end{bmatrix} \begin{bmatrix} \mathbf{H} \otimes \mathbf{G} & 0 & 0 & 0 \\ 0 & \boldsymbol{\sigma}_{\mathbf{b}}^{\mathbf{2}} & 0 & 0 \\ 0 & 0 & \boldsymbol{\sigma}_{\mathbf{l}}^{\mathbf{2}} & 0 \\ 0 & 0 & 0 & \boldsymbol{\sigma}_{\mathbf{e}}^{\mathbf{2}} \end{bmatrix}$$

where **G** is the 3 × 3 (co)variance matrix of random regression coefficients for the additive genetic effect of sire; $\boldsymbol{\sigma}_{\mathbf{b}}^{\mathbf{2}}$ is the genetic variance for effect of batch $[N(0,\sigma_b^2)]$.; $\boldsymbol{\sigma}_{\mathbf{l}}^{\mathbf{2}}$ is the genetic variance for the effect of litter $[N(0,\sigma_l^2)]$.; and $\boldsymbol{\sigma}_{\mathbf{e}}^{\mathbf{2}}$ is heterogeneous residual variance coefficient referred to each class of climatic factor$[N(0, \sigma_e^2)]$. The matrix **H** consisted of the combination between the pedigree-based numerator relationship matrix **A** with the genomic information **G** in order to consider animals with and without genomic information simultaneously.

The inverse of H was computed using the preGSf90 software (Aguilar et al., 2014) and defined by the formula:

$$\mathbf{H}^{-1} = \mathbf{A}^{-1} + \begin{bmatrix} 0 & 0 \\ 0 & \mathbf{G}^{-1} - \mathbf{A}_{22}^{-1} \end{bmatrix}$$

in which G^{-1} was the inverse of the genomic relationship matrix and A_{22}^{-1} was the inverse of the numerator relationship matrix for genotyped animals. A total of 60,000 Gibbs samples were generated, while discarding the first 10,000 as burn-in and thinning every 10 samples. Posterior means and standard deviations of the remaining 5,000 samples were used as estimates and error of for the (co)variance components.

2.3.2 Estimation of genetic parameters

The additive genetic (co)variance structures of individual animals for each climatic variable were:

$$\mathrm{Var} = \Phi\ G\ \Phi$$

where Φ was a matrix of Legendre polynomial functions calculated for each value of the considered climatic variable and G was the 3 × 3 (co)variance matrix of random regression coefficients for the additive genetic effect of sire;

From gRRM each animal *l* had a *nr* random regression coefficient solutions. For any particular trait of interest, the *nr* solutions were converted to genomic breeding values

(**GEBV**) to calculate the effect of each climatic variable on genetic evaluations and ranking sires based on the higher and lower thermal resistance. The heat-tolerance GEBV for animal *l* was obtained with the formula:

$$\mathrm{GEBV}_{\mathrm{lm}} = \Phi\hat{\mathrm{u}}_{\mathrm{l}}$$

where Φ is a row vector with the o*th* elements equal to the the *jth* orthogonal polynomial from the minimum to maximum range of the weather variable; $\hat{\mathrm{u}}_{\mathrm{k}}$ is the vector for the regression coefficient of the animal l.

Heritability was estimated using variance components calculated over the range of variation of each climatic variable as:

$$\mathrm{h}^2 = \frac{4*\sigma_s^2}{\sigma_s^2+\sigma_b^2+\sigma_l^2+\sigma_e^2}$$

in which h^2 was the heritability for a specific climatic variable range; σ_s^2 is the estimate of genetic variance of sire; $\sigma_b^2$and σ_l^2 are the estimates of variance of batch and litter effects, respectively; σ_e^2 was the estimated residual.

3. Results and Discussion

3.1 Characterization of climatic conditions

The summary of monthly mean temperatures and humidity are illustrated in Figure 1, which shows the trend of average, maximum and minimum temperature throughout the considered period of study.

Three different seasons were defined based on the trends presented in Figure 1: a cold season, including the days with an average daily temperature (Tavg) below 10°C; a mild season for days with a Tavg between 10 and 20°C; and a hot season, which included the days with a Tavg over 20°C. Thus, November, December as well as January to the first part of March were considered as cold period; The months of June to July was considered as warm season; The second part of March to May, as well as September and October were considered as months of comfort.

The coldest periods of winter occurred in December and January which were associated with a mean daily maximum and minimum temperatures around 3.2 and -5.3°C, respectively. Intermediate temperature values have been found for yearly months of April, May and October, in contrast with the extreme minimum temperatures that occurred during the period from January to February (values varied between -10.2 °C and – 8.5 °C). Then, temperature starts to increase over the spring season reaching the maximum value of 32.3°C in July.

In Figure 1 is also illustrated the average annual trend of relative humidity. RHavg was almost constant over the year (around 51.4 %) with the lower values observed during the spring season, ranging between 69.6 % in April and 70.8 % in May. A larger variation between both seasons was observed for RHmin. The pattern plotted in Figure 1 shows a great oscillation occurred between the cold months (57.6%) and the comfort-warm period

(49.6 %). For all THI indices the trends observed were very close to those estimated for corresponding temperatures, with low values in winter and high during the summer.

As already pointed before, the best climatic predictors and the thermal load interval for each analyzed trait were selected considering the highest coefficient of determination (R^2) obtained from the model 1 and included in model 2 to estimate the effect of heat stress on each analyzed trait.

All coefficients of determination values were small, indicating that only a small part of yield variation is explained by the weather variables and the other fixed effects. Table 3 reported that for BF the best predictor ($R^2 = 0.144$) was RHavg recorded in the period between 92-122 days of age. The highest coefficient of determination ($R^2 = 0.122$) was obtained from the same climatic variable considering the period 60 - 92 days for HA. On the other hand, the best fit for LD and ADG occurred for the 122-152 period d period ($R^2=0.026$ and $R^2=0.057$, for LD and ADG, respectively).

3.2 Genetic parameters for estimated traits

Although RRM previously have been used to model animal weight (live weight) in dairy and beef cattle (Meyer, 2000; Coffey et al., 2006; Bradford et al., 2016; Bohlouli et al., 2018), the use of this approach to assess the effect of environmental conditions on animal conformation or carcass quality trait are nonexistent in pigs. All the studies regarding pigs available in the literature, used the RRM to determine the genetic parameters as a function of heat load on growth traits or carcass weight (Zumbach et al., 2008b; Fragomeni et al., 2016a,b).

In this study, results obtained with RRM showed evidence of genotype × environmental interaction effects regarding heat stress. Genetic correlations are summarized as a heat map for each analyzed trait in Figure 3. Results support the general impression that selection genetic correlations for BF across different values of RHavg were larger than estimates for the other traits. For all traits considered in this study the genetic correlations were greatest for lower values of each climatic factor. This is in line with those observed from Zumbach et al., (2008), who evaluated the effects of heat stress on finishing weight for the commercial farms found a greatest genetic correlation (0.53) during the "cold" months; BF correlations were generally strong. In particular, the higher values (0.95-0.98) were displayed for a RHavg between 62 and 70. Correlations of similar magnitude were also estimated for HA (0.85- 0.99) between 60 % and 68% of RHavg tending to decrease with the increase of RHavg.

Conversely to the high values obtained for BF and HA, the weakest genetic correlation (less than 0.50) was found for LD and ADG at different values of Tavg. As showed in figure 3, LD and ADG had a strong genetic correlation (above 0.80) at values of Tavg less of 10°C while the range of Tavg between 15°C and 26°C had genetic correlation near to 0.4 and 0.6.

The heritability estimates from the current study indicated a possible genetic improvement by selecting for the direct genetic component of carcass quality traits in heat-stressed conditions. These findings were true for all carcass quality traits across the range of the respective climatic variable values except for ADG and HA, which showed the lowest heritability (Figure 2). The range of variation over the RHavg (0.15- 0.25) showed for HA was larger than those observed for ADG. Heritability of HA was equal to 0.19 for a range of RHavg between 62 and 68 and increased for an RHavg greater than 73%. In our case, ADG has been reported as a very low heritable trait but most constant within the range of Tavg. Results illustrated in figure 2 shows that ADG was most heritable ($h^2 = 0.16$) for low temperatures comprised between -7°C and 4°C to decrease until an average of heritability equal to 0.08 over the range of Tavg, in contrast with some studies previously reported in carcass quality traits of pigs (Clutter AC 2011; Cassady et al., 2002).

Generally, previous studies reported carcass traits as moderate to high heritable traits (Fragomeni et al., 2016a, b). Considering commercial populations, Zumbach et al. (2008b) founded low to moderate heritability of 0.14 and 0.28 for carcass weight in environments without and with heat stress, respectively. A moderate heritability for carcass weight during heat stress was indicated from Bradford et al., (2016) when in a study conducted of beef cattle reported values between 0.24 for weaning weight and 0.32 for yearly weight. Among all carcass merit traits BF is the most studied trait as it is related to overall carcass yield (Correa et al., 2007). However, to our knowledge, no literature was found presenting correlations between the effect of heat stress on pork quality traits.

The estimate for BF (0.28 – 0.33) was lower than the average heritability (0.43) of many previous studies reported by Ciobanu et al. (2011). Figure 2a shows a constant trend (~ 0.32) with a range of variation from 0.24 and 0.42 over the range of RHavg, reaching values around 0.30 and 0.34 for the extreme humidity conditions. Similar to those observed for BF, the trend reported for LD (0.19-0.31) was lower than the average (0.47) of many previous studies reported by Stewart and Schinckel (1991) and Miar et al., (2014) above 18°C and 19°C degrees of Tavg.

3.3 *Estimated breeding values from RRM*

As previously proposed in other studies regarding dairy and beef cattle (Bradford et al., 2016; Fragomeni et al 2016b) the application of a random regression model allows the estimation of genetic (co)variance components and breeding values over the whole trajectory of environment-dependent variable. The use of the same approach in the analysis of carcass quality traits in pigs are, to our knowledge, nonexistent. In this study, RRM was implemented in order to rank the sires based on the higher and lower thermal resistance from the EBV calculated for each particular trait of interest. The 2 groups of sires were distinct across the range of each climatic factor for all traits (Figure 4) based on the intercept term of the random regression model under standard environmental conditions. The 10 sires with the largest EBV were important for producers because would be considering these animals for the design of a breeding program to obtain a better meat quality in swine population.

Although all traits have shown difference between extreme environmental conditions, the ADG was the one that best reflected the effect of heat stress conditions on animal performance. In this study, the antagonistic relationship between level of production and response to heat stress was evidenced by the reduced tolerance to environmental thermal stress associated to sires with higher values of EBV (higher group). Results in figure 4 shows that considering the higher group of sires, the drop of ADG ranged between 0.550 to 0.535 kg/d over the range of temperature. Accordingly, among sires of lower group there was a higher tolerance to extreme temperature conditions associated to a stronger variability corresponding to lowest values of temperature (between -7°C and 9°C degrees).

A similar pattern was obtained for BF trait. Animals most tolerant were those with the lower value of EBV and a most constant trend over the considered range of RHavg, showing a difference between the higher and lower range of RHavg equal to 1,5 mm of BF.

The drop observed for higher values of each climatic factor revealed that animals presumably raised during the summer period were those with a stronger decline in ADG and BF unlike animals fattened over the cold period. Lower carcass fatness at slaughter connected to the decline in feed intake is generally reported in heat- stressed pigs (Le Dividich et al., 1998). Thus, according with Le Bellego et al., (2002) and Renaudeau et al., (2011) the decrease in growth rate associated with thermal stress could be primarily a result of a decline in feed intake, although a slight increase in feed conversion ratio at very high-temperature level.

Little research has been conducted to evaluate the impact of stressors on the variation of measures of carcass composition. However, it is already known that during heat stress, feed intake is decreased in order to reduce the heat production associated with the digestion and metabolism of nutrient (Ross et al., 2015). This suggests that differences in ADG and BF observed among animals under different environmental conditions in this study, could be mainly related to the management of farmed animals strictly linked to the achievement of the slaughter weight required by the market.

In order to better understand the flow of animals along the year and investigate patterns of exposure to thermal stress Table 2 was built. Based on the birth and harvest date, was defined the period of the animals into the feedlot. Animals considered in this study were generally slaughtered after six or seven months into the commercial farms. The optimal body weight market for pigs was

primarily determined by their compositional growth and feed efficiency during the late finishing stage of body weight growth. However, the growth rate of pigs due to different environmental period under the commercial farm could determine that animals were not ready for slaughter at the same time.

Data reported in Table 2 show that pigs born between March and April reached their target body weight market in September and October, after seven or eight months into the commercial farm. Animals fattened during this period were exposed to warmer environmental conditions, when high temperatures may slow down the growth rate and increased the weight variation.

Similar to feed intake, ADG has a decreasing response during the thermal load and is affected by the animal's body weight with heavier pigs more susceptible to heat stress than lighter ones (Renadeau et al., 2011). In our study, the animals belonging to the higher group reduced their ADG from 0.55 to 0.53 passing from -7°C to 26°C. This sensitivity to warmer temperature suggests that during hot summer months (when feed intake is reduced) pigs did not consume sufficient amount of food for normal lean gain. Heavy losses in weight over the summer season are recovered during the following months, when the animals recovered what they had lost and made all their additional growth. Thus, pigs included in this group grew more than pigs fattened during the winter period. Table 2 shows that animals born during the winter period of November and December were able to achieve optimal pig growth around 5 and 6 months of fattening, before the start of the warm season.

This explains also the increase in LD in both, higher and lower group of sires over the range of Tavg. The increase of LD was essential constant and equal to 3 mm of LD passing from -7 °C to 26°C of Tavg, in contrast with the common observation on heat-stressed finishing pigs (Cruzen et al., 2015; Ross et al., 2015). In addition, the apparent increase of LD observed under higher Tavg could be related to the selection applied from the pork industry over the years for pigs that grow leaner. The aim in pig breeding is to select with a greater genetic capacity for rapid lean growth up to slaughter weight. Schinckel et al., (2008) studied the compositional growth of two genetic populations differing in carcass lean and fat tissue growth concluded that lean-gain pigs had greater relative rates of protein deposition and lesser rate of lipid deposition per kg of body weight than low lean-gain pigs.

As regards the HA, the animals included in the higher group had a different age at slaughter passing from the lowest to the highest values of RHavg. In correspondence of RHavg values, between 62 and 72, the age at slaughter ranged from 176 to 206 days while for humidity values between 73 and 83 the range of variability of the age at slaughter tended to decrease from 191 to 161 days. On the other hand, the animals included in the lower group, showed a similar trend in age at slaughter for both low and high RHavg values.

The EBV for the 20 sires were also estimated based on the reaction of animals to the environmental conditions (Figure 5). For BF the lower and higher groups of animals showed a trend opposite to those observed in figure 4. The 10 sires with higher EBV seemed to be most tolerant to all the range of RHavg than higher group which was most sensible to values of RHavg comprised between 74 and 83. As already pointed before (Figure 4) the pattern of variation of LD was in contrast to those described for BF and ADG. In this case, the higher group was those most sensible to the lower values of Tavg (between -7°C and 2°C degrees) to increase in correspondence of the extreme higher values of Tavg. Based of reaction of animals to environmental conditions ADG showed the most constant trend for the lower group. Most productive animals were most resistant to coldest temperatures condition but recorded a larger drop for higher values of Tavg. As regards the HA, the separation between higher and lower groups was more evident over the considered range of RHavg. However, figure 5 shows that for the higher group the age at slaughter tended to increase with the increase of RHavg values while animals included in the lower group tended to be slaughtered between 170 and 160 days.

Differences reported in this study between no thermal stress and maximum thermal stress scenario would have a large economic impact for producers in swine production, influencing the meat quality and consequently the profit of pork industry. The methodology proposed in this study using weather information to identify heat tolerant animals could be a useful tool to improve the production system and implement the selection programs. Ideally, the use of this approach represents an optimal breeding strategy to improve heat tolerance in relation to the farm resources (including nutrition, management, and investment capacity). Farming systems with enough resources to ensure high productivity will benefit more from including heat tolerance in the breeding programs of individuals that are resistant to extreme conditions. On the other hand, due to the antagonism between heat tolerance and productivity, farms with scarce resources will take more advantage from individuals most adaptable to adverse environmental conditions.

4. Conclusions

A random regression model including genomic information was used to evaluate the effect of heat stress on carcass quality traits of crossbred pigs. Results show evidence of different genetics underlying phenotypes in different temperature and humidity conditions, suggesting the existence of genotype × environment interaction. Differences reported in this study among the analyzed carcass quality traits would have a large economic impact for producers in swine production. Backfat and average daily gain were those that showed the best fit, revealing a potential impact on meat quality and consequently on the profit of pork industry. The combination between heat tolerance and records for all these traits into one index could be considered a useful tool to identify most tolerant sires and mitigate seasonal losses in a commercial crossbred swine population. Traits under heat stress seems to be more heritable than under mild conditions, indicating that the identification and selection of the most resistant animals is possible in order to implement the selection programs. However, further research is needed for the heat tolerance of swine to overcome the complexity of selection of heat tolerant animals, due to heat stress response and the antagonism between heat tolerance and productivity. Actually, the best solution seems to combine the breeding strategies with different resources of production systems.

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Table 1. Descriptive statistics for carcass quality traits in crossbreed pigs (n= 126,052)

Item	Mean	SD[1]
Age, days		
At weaning	19.3	2.00
On Test	78.3	17.5
Harvest	178	10.6
Carcass traits		
Backfat, mm	18.7	4.11
Loin Depth, mm	67.3	7.03
Average Daily Gain, kg/d	0.54	0.12

[1] SD: Standard deviation

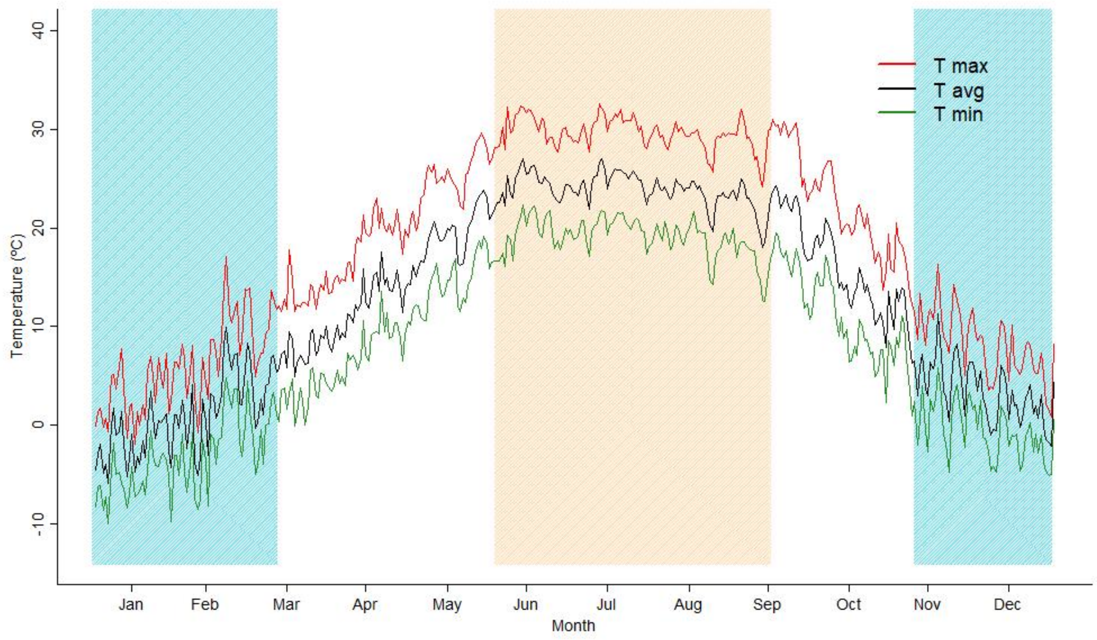
Table 2. Investigate year period of exposure to thermal stress based on the flow of animals along the year between the month of birth and harvest month.

Month	Month of Harvest												
of Birth	**Jan**	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**	**Total**
Jan						1059 12.5 %	6425 75.9 %	978 11.6 %					8462 100 %
Feb							2807 21.8 %	8892 69 %	1185 9.2 %				12884 100 %
Mar								1331 12.8 %	6555 62.9 %	2534 24.3 %			10420 100 %
Apr									1555 10.3 %	12617 83.3 %	967 6.4 %		15139 100 %
May										6139 40.4 %	7804 51.3 %	1260 8.3 %	15203 100 %
Jun	7 0 %										4055 28.8 %	10004 71.1 %	14066 100 %
Jul	844 19.6 %											3468 80.4 %	4312 100 %
Aug	1717 16.9 %	7284 71.8 %	1150 11.3 %										10151 100 %
Sep	1 0 %	2870 24.4 %	6587 56.1 %	2285 19.5 %									11743 100 %
Oct			2116 15.3 %	10727 77.4 %	1014 7.3 %								13857 100 %
Nov				475 17.4 %	2032 74.4 %	223 8.2 %							2730 100 %
Dec					1474 20.8 %	4801 67.8 %	809 11.4 %						7084 100 %
Total	2569 2 %	10154 8.1 %	9853 7.8 %	13487 10.7 %	4520 3.6 %	6083 4.8 %	10041 8 %	11201 8.9 %	9295 7.4 %	21290 16.9 %	12826 10.2 %	14732 11.7 %	126051 100 %

Table 3. R^2 for different heat load functions depending on the climatic variable (T avg, RH avg and THI avg) and considered period[1].

Item	Days		
	60-92	92-122	122-152
Carcass Backfat, mm			
T avg (°C)	0.134	0.125	0.136
RH (%)	0.139	**0.144**	0.125
THI avg	0.135	0.125	0.135
Carcass Loin Depth, mm			
T avg (°C)	0.015	0.022	**0.026**
RH (%)	0.011	0.017	0.011
THI avg	0.014	0.022	0.014
Average Daily Gain, kg/d			
T avg (°C)	0.040	0.055	**0.057**
RH (%)	0.041	0.048	0.041
THI avg	0.041	0.055	0.041
Harvest Age, days			
T avg (°C)	0.099	0.118	0.118
RH (%)	**0.122**	0.098	0.101
THI avg	0.099	0.115	0.099

[1] R^2: Coefficients of determination; Tavg = daily average temperature (°C); RHavg= daily average relative humidity (%); THIavg= daily average values of temperature – humidity index (°C); The values in bold indicate the highest coefficient of determination obtain from each run of model 1.



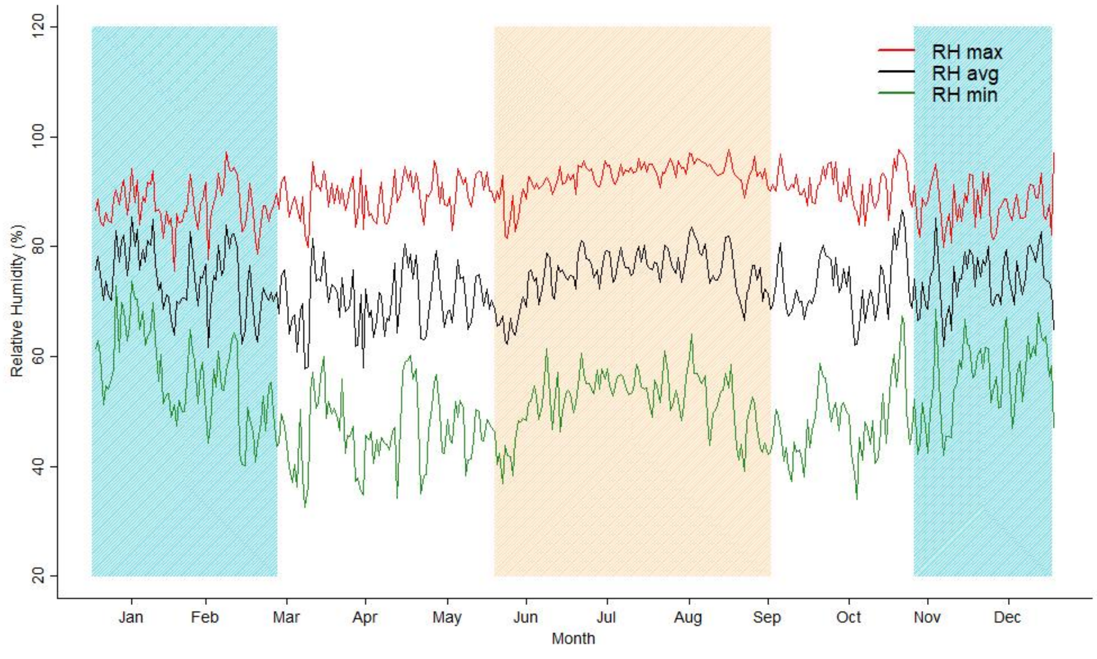


Figure 1. Average values of daily maximum (Tmax), average (Tavg), and minimum (Tmin) temperatures, and daily maximum (RHmax), average (RHavg), and minimum (RHmin) relative humidity through the year for years 2015 to 2019 in the weather stations. Shadowed regions represent hot (top-left to bottom-right lines) and cold (bottom-left to top-right lines) seasons.

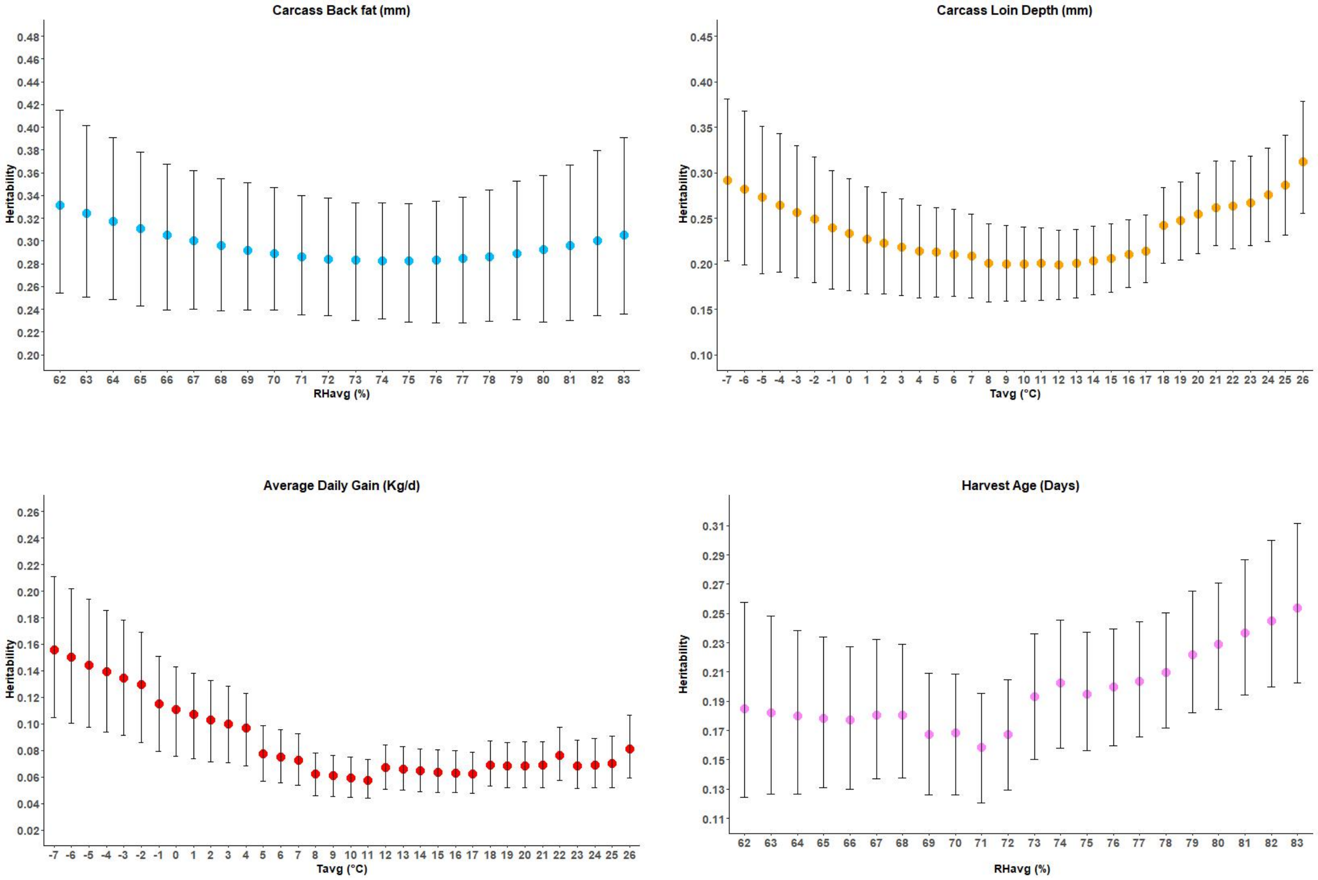
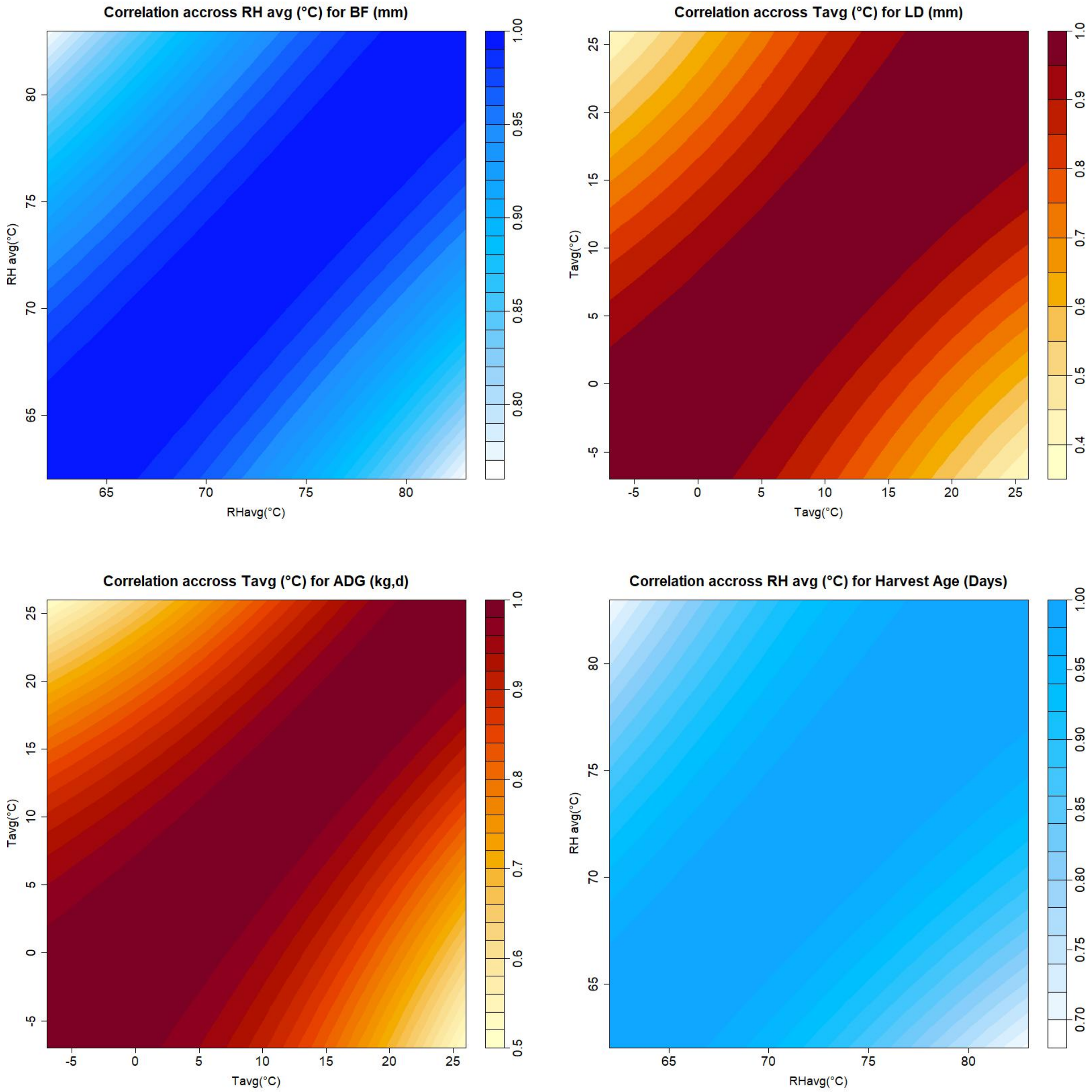


Figure 2. Heritability estimates for the three carcass quality traits and harvest age of animals over the range of the respective climatic variable. Tavg = daily average temperature; RHavg= daily average relative humidity.


Correlation accross RH avg (°C) for BF (mm)

Correlation accross Tavg (°C) for LD (mm)

Correlation accross Tavg (°C) for ADG (kg,d)

Correlation accross RH avg (°C) for Harvest Age (Days)

Figure 3. Additive genetic correlations estimated with the genomic random regression model [2] for the corresponding climatic variable and carcass quality trait combinations. Daily average temperature (Tavg, °C); Daily average relative humidity (RHavg, %); carcass backfat (BF, mm); Loin depth (LD, mm) and carcass average daily gain (ADG, Kg/d).

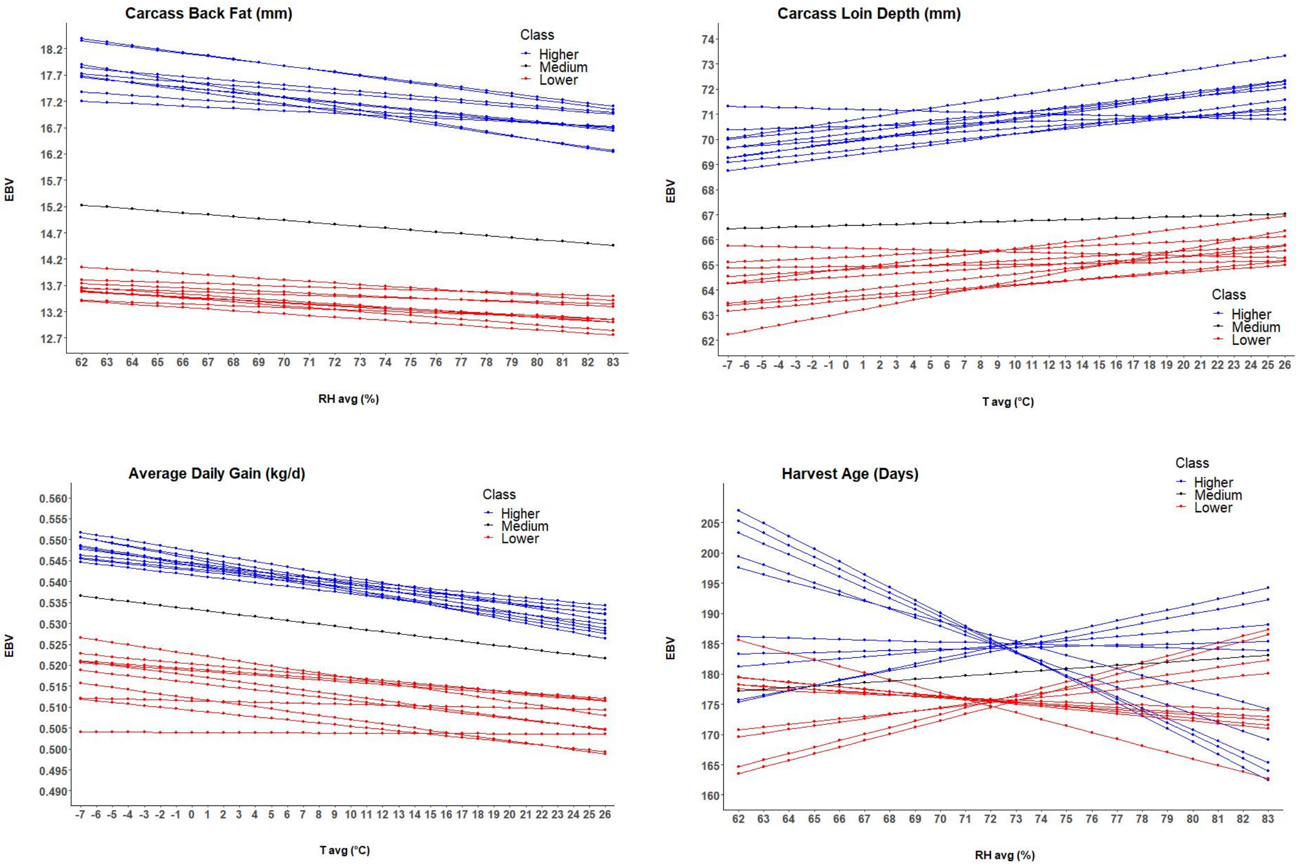


Figure 4. Reaction norms for the twenty sires showing the highest and lowest estimated breeding values (EBV) for the intercept term of the random regression model (performance under standard environmental condition). Tavg = daily average temperature; RHavg= daily average relative humidity.

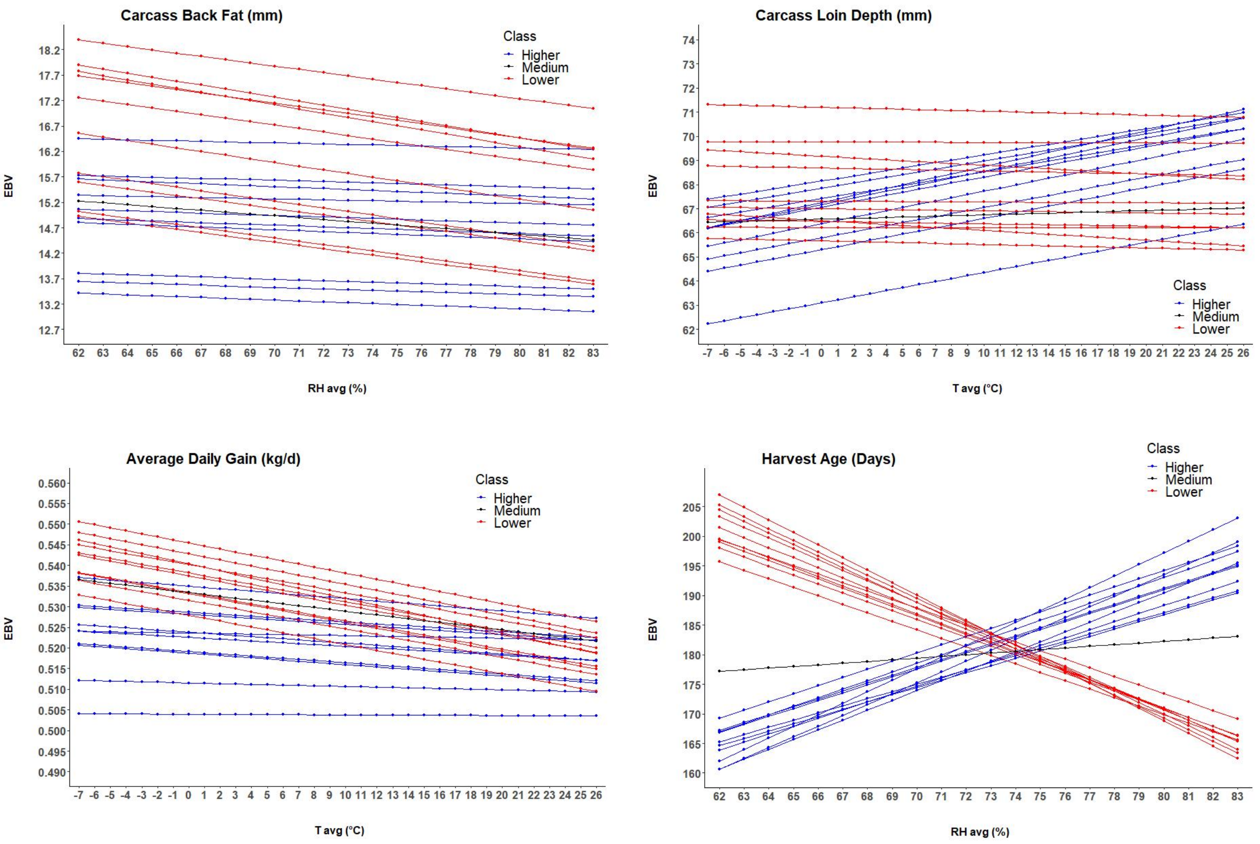


Figure 5. Reaction norms for the twenty sires showing the highest and lowest estimated breeding values (EBV) for the slope term of the random regression model (change in performance due to change in environmental conditions); Tavg = daily average temperature; RHavg= daily average relative humidity.

Conclusions

The analysis of direct and indirect climate change impacts on livestock production are clear and widely documented. Global climate changing is expected to determine a substantial decrease on farmer income because of its effect on animals performance and well-being. The genetic selection of less sensitive individuals to both heat and/or cold temperatures could represent a main strategy to mitigate the effect of climate on production performances. A problem related to the thermal tolerance is the complex phenomenon of antagonism between adaptability and production.

In order to investigate the impact of climate conditions on production traits we have analyzed three different scenarios of livestock species: dairy sheep, beef calves and pigs. Results obtained in these studies revealed the sensibility of all considered species to the thermal conditions they are exposed.

The study reported in chapter 2 on Sarda dairy ewes showed that the lactose content was the milk component more strongly affected by the THImax effect. The slight reduction of protein concentration observed with the highest THImax values probably determined the general worsening of the MCP. However, the unclear pattern of protein observed during this period needs to be deeply investigated for better understand the correlation with MCP during summer period. As observed in other studies on dairy sheep breeds, the overlapping of climatic conditions, lactation stage, and parity made it difficult to properly assess the effect of climate on milk production traits i under Mediterranean semi-extensive and extensive farming conditions.

Results in Chapter 3 allows us to identify the average daily temperatures (Tavg) as the best predictor of the relationships between the weight trait and climatic variables. A wide comfort zone, between 0.3 °C and 25°C in which animals could express their highest production ability has been identified. It has been observed that if calves were subjected to constant temperatures (above threshold) the effect of heat stress would be twice as much as the one of cold stress. However, under the climatic conditions where these animals are fattened cold stress results in largest loss of weight. Thus, the impact of heat or cold stress on these calves could change depending on the general climate pattern.

In chapter 4, the genetic components of thermal response in male calves of the Spanish local breed Avileña -Negra Ibérica were estimated. Among the two alternative models, the cubic equation provided a better fit of the analyzed data. In this population, genotype × environment seemed to be small or moderate regarding weight achieved under a wide range of temperatures. The small genotype × environment interaction found in our analysis is probably related with the adaptability of this breed to adverse environments. These animals are characterized by a high adaptability to arid conditions and by a strong capacity to travel long distances in search of pasture. Thus, the adaptive advantages to extreme temperature conditions of these animals could result in a lower genotype × environment interaction.

A similar study was described in chapter 5 on a population of commercial pigs including genomic information in a random regression model. Results obtained with RRMs showed evidence of genotype × environmental interaction effects regarding heat stress. This suggested that the existence of a higher genotype × environment interaction is probably due to the high selection this species has been subjected. Genetic components were estimated in order to establish main features of trait deviations, to consider heat tolerance in breeding programs.

Differences reported among the analyzed traits would have a large economic impact for livestock producers. Results of this thesis suggest that farmers should take into account the variability of the environmental scenarios when they have to plan the investments in the animal production sector. The implementation of adaptation measures is really important especially in areas subjected to extreme climatic events. In order to implement the selection programs of livestock species, the geographic location information of animals should be provided to combine thermal tolerance for all of these traits into an index. The breed associations and producers could then use these selection tools for the identification of the individuals more resistant to extreme environmental conditions and minimize production costs improving the quality of the final product. Moreover, the measures of adaptation and mitigation should be supported by adequate quantitative scientific studies on future environmental conditions.