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Coping with Climate Change: Understanding the Genetic Makeup for Adaptation in Sheep under Different Climate Conditions

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COPING WITH CLIMATE CHANGE: UNDERSTANDING THE GENETIC MAKEUP FOR ADAPTATION IN SHEEP UNDER DIFFERENT CLIMATE CONDITION

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ABCG1	ATP binding cassette subfamily G member 1
ABCG2	ATP binding cassette subfamily G member 2
ACTH	Adrenocorticotropic hormone
ASIP	Agouti signaling protein
BMCs	Blood mononuclear cells
BTN1A1	Butyrophilin subfamily 1 member A1
BTNL2	Butyrophilin like 2
BTV	Bluetongue virus
CD40	Cluster of differentiation 40
CPM	Log counts per million
CRYL1	Crystallin lambda 1
CSN1S1	Casein alpha s1
CSN2	Casein beta
DCT	Dopachrome tautomerase
DDC	Dopa decarboxylase
DIO2	Deiodinase iodothyronine type II
DNA	Deoxyribonucleic acid
DNAJC8	DnaJ heat shock protein family member C8
DNAJC18	DnaJ heat shock protein family member C18
dsDNA	Double-stranded DNA
ESR1	Oestrogen receptor 1
FAM107B	Family with sequence similarity 107 member B
FBXO11	F-box protein 11
FRET	Fluorescence resonance energy transfer
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
GMEB2	Glucocorticoid modulatory element binding protein 2
GNRH1	Gonadotropin releasing hormone 1
GPNMB	Glycoprotein non-metastatic melanoma protein B
GOT1	Glutamic-oxaloacetic transaminase
GSTCD	Glutathione S-transferase C-terminal domain containing
HCRT	Hypocretin neuropeptide precursor
HSP	Heat shock proteins
HSP70	70kDA heat shock proteins
HSP90	90kDA heat shock proteins
HSP90AA1	Heat shock protein 90 alpha family class A member 1
HSP90AB1	Heat shock protein 90 alpha family class B member 1
HSPA12A	Heat shock protein family A member 12A
HSPA13	Heat shock protein family A member 13
HSPA4	Heat shock protein family A member 4
HSPA8	Heat shock protein family A member 8
HTR1B	5-Hydroxytryptamine receptor 1B
HTR4	5-Hydroxytryptamine receptor 4
HTR5A	5-hydroxytryptamine receptor 5A
HWE	Hardy–Weinberg equilibrium
ICAM-1	Intercellular adhesion molecule 1
IGF1	Insulin like growth factor 1
IL1R1	Interleukin 1 receptor type 1

IL1R1_2	Interleukin 1 receptor type 1
IL10RB	Interleukin 10 receptor, beta subunit
IL2	Interleukin 2
IL33	Interleukin 33
IL33A	Interleukin-23 subunit alpha
IL6	Interleukin 6
INPP5B	Inositol polyphosphate-5-phosphatase B
IRF4	Interferon regulatory factor 4
KIT	Pronto-oncogene, receptor tyrosine kinase
KRTAP6-1	Keratin associated protein 6-1
LEP	Leptin
LPS	Lipopolysaccharide
L-DOPA	Levodopa
MAPRE1	Microtubule associated protein RP/EB family member 1
MLANA	Melan-A
MST1	Macrophage stimulating 1
MYO5A	Myosin VA
NBEA	Neurobeachin
NCAD	Cadherin 2
OCA2	OCA2 melanosomal transmembrane protein
PAM	Peptidylglycine alpha-amidating monooxygenase
PAMP	Pathogen-associated molecular pattern
PAX3	Paired box gene 3
PCK	Phosphoenolpyruvate carboxykinase
PECAM1	Platelet and endothelial cell adhesion molecule 1
PGs	Prostaglandins
PHC3	Polyhomeotic homolog 3
PIC	Polymorphic information content
PIK3C2B	Phosphatidylinositol-4-phosphate 3-kinase catalytic subunit type 2 beta
PLCB4	Phospholipase C beta 4
PLP1	Proteolipid protein 1
PLXCN1	Plexin C1
PMEL	Premelanosome protein
POMC	Proopiomelanocortin
PPARG	Peroxisome proliferator-activated receptor gamma
PPI	Protein-protein interaction
PPRV	Peste des petits ruminants virus
PRKG1	Protein kinase CGMP-dependent
RARα	Retinoic acid receptor alpha
RTN1	Reticulon 1
SAM	Sympathetic-adrenal-medullary
SFRP1	Secreted frizzled related protein 1
SLC	Solute carrier
SLC24A4	Solute carrier family 24 member 4
SLC24A5	Solute carrier family 24 member 5
SLC40A1	Solute carrier family 40 member 1
SLC45A2	Solute carrier family 45 member 2
SNPs	Single-nucleotide polymorphisms
SOCS2	Suppressor of cytokine signaling 2
SOCS3	Suppressor of cytokine signaling 3

SOD	Superoxide dismutase
SOD1	Superoxide dismutase type 1
SOD2	Superoxide dismutase type 2
STAT_PIAS3	Protein inhibitor of activated STAT 3
STAT1	Signal transducer and activator of transcription 1
STAT3	Signal transducer and activator of transcription 3
T_{db}	Dry bulb temperature
Th1	T-helper 1
Th2	T-helper 2
TLR2	Toll like receptor 2
TLR5	Toll like receptor 5
TLR7	Toll like receptor 7
TLR8	Toll like receptor 8
TR	Thyroglobulin
TRPM1	Transient receptor potential cation channel subfamily M member 1
TYR	Tyrosinase
TYRP1	Tyrosinase related protein 1
USP19	Ubiquitin specific peptidase 19
USP43	Ubiquitin specific peptidase 43
VNN1	Vascular noninflammatory molecule-1

1. INTRODUCTION

1.1. Research background

Over the last several decades, increasing livestock population and production has primarily meant increasing output to keep up with the world's ever-increasing demand for food. Genetic selection and the introduction of internationally proven breeds with better genetics are our primary strategies in order to increase production, complemented by the development of optimal management systems to maximize genetic potential. However, in recent years, there has been a shift in the livestock production system's emphasis towards a stress-free system, driven by increased awareness of animal welfare. Simultaneously, the persistent trajectory of global climate change adds new challenges to animal welfare issues, such as the discomfort animals feel as a result of environmental challenges, particularly heat stress. The objectives of livestock production have become more complex: mitigating the adverse impacts of climate change on livestock especially heat stress, while maintaining or even increasing growth and productivity, as the demand for animal-based food continues to rise.

There is little doubt that climate change is the most pressing environmental issue of our era, with extensive and significant adverse impacts, and appears unlikely to become less severe in the near future. Additionally, the operation system of livestock production will become more complex due to the substantial challenges posed by factors such as increasing urbanization, changing lifestyles, limited land availability, limiting bioresource, and growing populations. Not to mention that both environmental sustainability and climate change are profoundly affected by livestock production, which has led to a long-term discussion about the apparently paradoxical relationship between the two. The redistribution of energy resources towards adaptation mechanisms may explain why livestock productivity is so negatively impacted by climate change. But the production activities, including enteric fermentation and manure fermentation, as well as the greenhouse gas pool, are a major contributor to the current climate change. This is especially true in ruminants. It is, therefore, necessary to establish sustainable livestock production methods that are both climate change adaptable and less damaging to the environment.

There are direct and indirect ways in which climate change affects livestock farming. The direct effects on livestock biochemical regulation include changes in feed intake and hormone levels as well as alterations in energy metabolism and utilisation and the accumulation of reactive oxygen species. This impairs the reproductive cycle, immunity,

growth rate, milk quality and quantity, and production in the long run. Some of the indirect effects of climate change on livestock farming resources include changes in pasture quality and quantity as a result of altered seasonal patterns, disruptions to ecosystems, the rapid spread of parasites and diseases, and reduced water availability. Feed and nutrition management, modern housing with precision technology, and breeding strategies are some of the measures taken to reduce, the negative effects of climate change on livestock production. The first two options, however, require substantial expenses of capital and consistent effort. Thus, a potential long-term solution to the problem of livestock adaptation could be to breed and select animals that are more resistant to the effects of climate change.

Thus, understanding the genetic architecture and molecular basis of adaptation traits is crucial for breeding and selecting animals with heat resistance. These traits are regulated in complex mechanisms involving extensive gene networks. Fortunately, the advancement of genomic technologies in livestock facilitates our understanding of the genetic framework for climate change adaptability.

The objective of this doctoral research was to use polymorphic study and gene expression study approach to contribute to the comprehension of the genetic architecture of heat stress (HS), an adverse consequence of climate change in sheep *(Ovis aries).* Sheep were the primary subjects of this study because limited knowledge is known about their adaptability to HS. Additionally, sheep are widely distributed across the world and are considered one of the most adaptable livestock species, making them an ideal model for this type of research and future livestock production sustainability. In addition, sheep are an excellent and potentially useful livestock resource for dealing with the anticipated climate change, especially in developing countries.

1.2. Research aims

This doctoral research is conducted in three distinct phases with the following objectives for each phase.

i. To study polymorphism of ovine genes associated with heat resistance traits across various sheep breeds from different climatic conditions using single nucleotide polymorphisms (SNPs) markers.

ii. To detect seasonal relative expression levels of heat stress and immunity associated genes in different sheep breeds reared in Hungary's environment using quantitative real time polymerase chain reaction (qRT-PCR) method.

iii. To investigate the molecular mechanisms underlying thermoregulation in white-coated and black-coated Hortobágyi Racka sheep using RNA sequencing (RNA seq) method.

2. LITERATURE REVIEW

2.1. Climate change

Climate change refers to the variations in the climate system, including the atmosphere, biogeochemical cycles, land surface, ice, and the biotic and abiotic elements of the Earth. The climate changes are characterized by elevated temperatures, greater variability in precipitation, and an increased occurrence of extreme weather events. The environment is greatly affected by the occurrence of global warming, which is defined as a widespread increase in average global temperatures (AHMED, 2020; CHENG et al., 2022).

Changes in global climate patterns have been accelerated by the overproduction and atmospheric accumulation of greenhouse gases (GHG) (CRIPPA et al., 2021). According to the global greenhouse gas emissions report, there was a little increase of 0.4% in GHG emissions per capita in 2022. From 1990 to 2022, emissions increased 8.3% overall, with a per capita increase from 6.24 t CO_2eq/cap to 6.76 t CO_2eq/cap. Over the last hundred years, global temperatures have climbed 0.74 °C, while atmospheric carbon dioxide levels have surged from 278 ppm before industrialisation to 415 ppm now. Worldwide temperatures are expected to keep rising at a concerning rate throughout the 21st century, according to the IPCC (FAO, 2013; NASA, 2023).

Since 1975 onwards, the average global temperature has increased at a pace of 0.15-0.20 °C every decade (MALHI et al., 2021), with the most recent press released by World Meteorological Organization (WMO) (2024), saying that Summer 2024 was the hottest year on the record, with global average temperature from January-September of 1.54 (±0.13) °C above pre-industrial level. If appropriate measures are not implemented, this situation could worsen. According to the probabilistic projections of the IPCC's climate sensitivity range, this condition is projected to increase by 2 °C by 2100 and 4.2 °C by 2400 (IPCC, 2013). Climate change can be categorized into natural or anthropogenic causes. However, compelling evidence indicates that the primary factor behind the observed warming in the past 50 years is human activity, including livestock farming (Figure 1) (YADAV and DEVI, 2019).

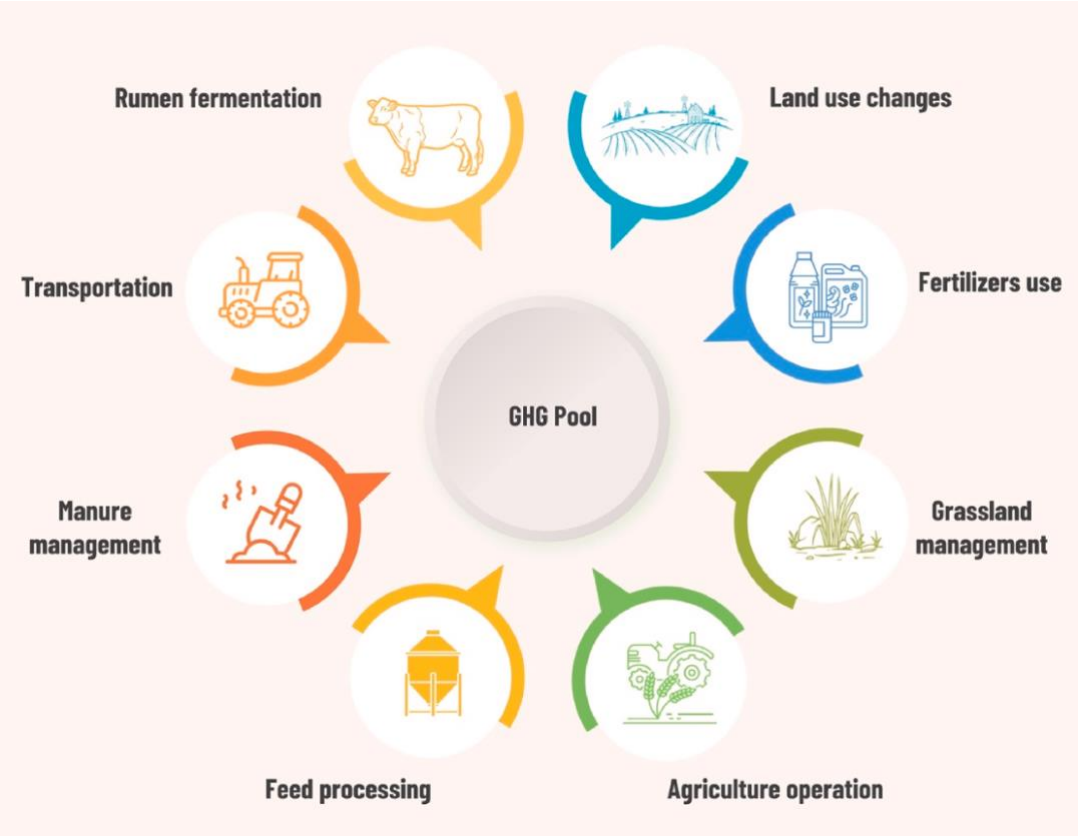

Figure 1. The role of the livestock industry in the greenhouse gas emissions pool (ASTUTI et al., 2024)

About 60% of all agricultural greenhouse gas emissions, mostly nitrogen oxide and methane, are generated by livestock farming (Figure 2). An estimated 14.5% of all human-caused greenhouse gas emissions come from ruminants, with 7.1 Gt CO_2 eq/yr in emissions (KLEPPEL, 2020). Also, a large portion of the greenhouse gas emissions from livestock come from ruminants, such as dairy and beef cattle. Livestock production (45%) and enteric fermentation (39%), both of which contribute to climate change, are realised by the livestock industry (GERBER et al., 2013). Additional sources of GHG emissions include shifts in land use, the production and distribution of goods derived from animals, and other similar activities (O'MARA et al., 2011). Direct and indirect emissions are influenced by factors such as animal species, population size, treatment methods, manure storage facilities, and land use management practices (KLEPPEL, 2020). The main ways that methane is directly released into the atmosphere are through respiration, excretions, and enteric fermentation (JUNGBLUTH et al., 2001). Also adding to indirect emissions are transportation, processing livestock products, and farming management methods. The

release of carbon into the atmosphere is primarily caused by indirect emissions in the cattle sector, rather than by direct emissions (LESSCHEN et al., 2011).

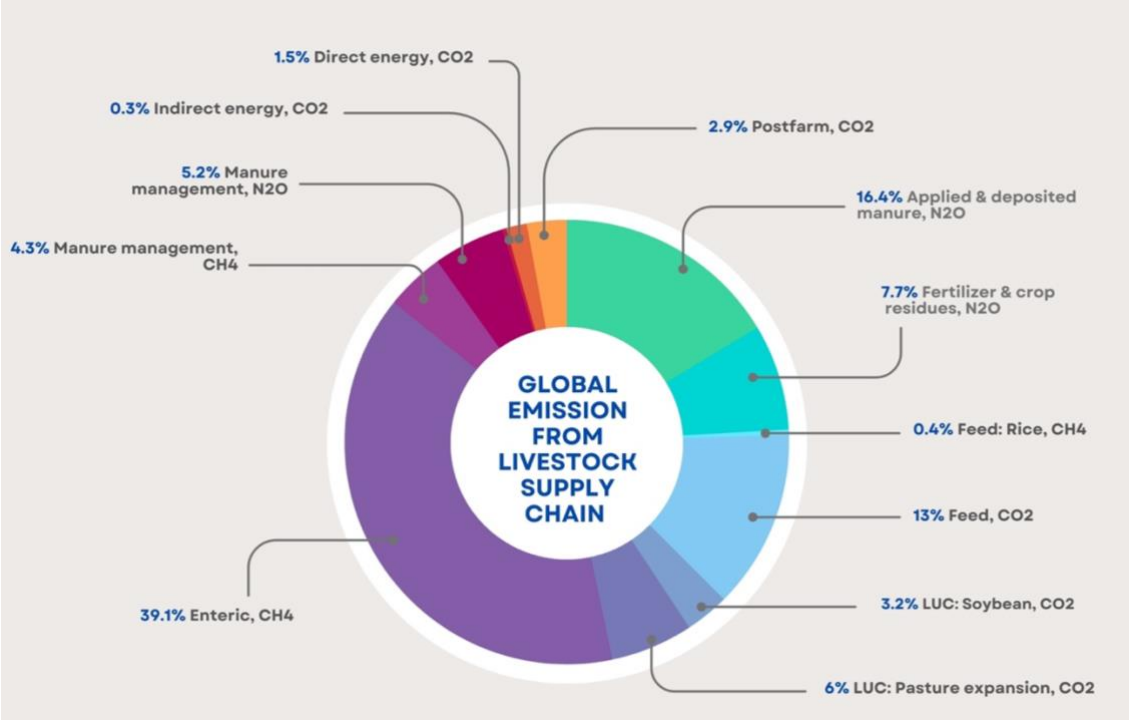

Figure 2. The impact of the livestock supply chain on world emissions. LUC =land usage change (ASTUTI et al., 2024)

Climate change is anticipated to have a significant impact on agriculture through several factors such as alterations in precipitation, temperature, carbon dioxide fertilization, climate variability, and surface water runoff. In addition to diminishing production capacity, climate change can accelerate the extinction of several species and the degradation of their habitats (KARIMI et al., 2018; MURRAY-TORTAROLO and JARAMILLO, 2019). Alterations in precipitation patterns lead to both droughts and floods, hence reducing agricultural productivity and food security. This will result in soil degradation, leading to nutrient leaching, erosion, and compaction (SALEEM et al., 2024). Rapid alterations in climate and extreme climatic events can alter species' thermal ecologies, reducing generation times, elevating metabolic demands, modifying distribution patterns, and changing seasonal phenologies, thereby affecting the intensity of species interactions and leading to species extinction (PFENNING-BUTTERWORTH et al., 2024).

In livestock farming, it directly affects by altering biochemical and physiological processes within their bodies. This can result in decreased feed consumption, changes in

energy utilization, reduced productivity and reproductive performance, disruption of immunity, and in severe cases, even mortality. Climate change has an indirect adverse effect on the feed supply in terms of both its quality and quantity, as well as on water availability. Additionally, climate change also affects the distribution, occurrence, and intensity of disease agents (GODDE et al., 2021; CHENG et al., 2022). For example, modelling research in UK found that when the consequences of climate change are considered alongside disease conditions on farms, the losses of net profit could be double for dairy cattle farms and multiply by six for beef farms (SHRESHTA et al., 2020).

The economic impact of livestock production in a climate change scenario has been analysed and estimated world widely. Heat stress is predicted to cost the U.S. livestock industry between $1.7 and $2.4 billion per year (ST-PIERRE et al., 2023). In case of Turkey, dairy cattle farms can expect to see a 10-50% rise in costs by 2044 due to climate change (KOÇ & UZMAY, 2019). In China, another study using five models predicted that temperatures rise between 0.85 °C and 6.73 °C and precipitation will increase between 4.98% and 15.47% from 2041 to 2060. The study also estimates losses in net livestock revenue, which range from −852 to −6700 USD (FENG et al., 2020).

2.2. Heat stress impacts on livestock

HS is an important aspect of climate change that has a severe influence on livestock production. SAWYER and NARAYAN (2019) define HS as "*any combination of environmental variables resulting in temperatures higher than the temperature range of the animal's thermoneutral zone*". This can be further explained as the accumulation of the external forces (which could be a combination of temperature, humidity, wind speed, and solar radiation) that are imposed upon an animal, resulting in an increase in body temperature and a corresponding physiological reaction. HS occurs when an animal's internal heat output exceeds its ability to dissipate heat (BERNABUCCI et al., 2010; HOFFMANN et al., 2020).

Homeothermy is maintained when the heat generated through metabolism, together with the heat absorbed from the surroundings, is equivalent to the dissipated heat flow from the animal to the environment. Within the thermoneutral zone, homeothermy is maintained through the processes of heat production and heat loss, including conduction, radiation, convection, and evaporation (LUZ et al., 2015). Animals must maintain their homeothermy to ensure optimal behavioural and physiological functioning. Each species

of animal has distinct thermoregulatory mechanisms, resulting in varying degrees of production decline when subjected to increased temperatures. In the process, endocrine responses play a key role in regulating the adaptation of animals to HS. Homeostasis relies on the proper functioning of the Hypothalamic-Pituitary-Adrenal (HPA) axis (NIYAS et al., 2015). Activation of the HPA axis in response to HS triggers the release of cortisol, a well-known indicator of stress, and also increases the levels of circulating glucose, which is crucial for managing the effects of heat stress. Cortisol can enhance the sensitivity of the HPA axis to future stressors by inhibiting the production of pro-inflammatory cytokines through negative feedback. Endocrine secretions can impact the development and function of Heat Shock Proteins (HSPs) in cells and tissues as a defensive mechanism. Additionally, HSPs can also exert control over the immune system. During heat stress, there is a complex interaction between the endocrine secretions, HSPs, and the immune system (JOY et al., 2020a; CHEN et al., 2023a).

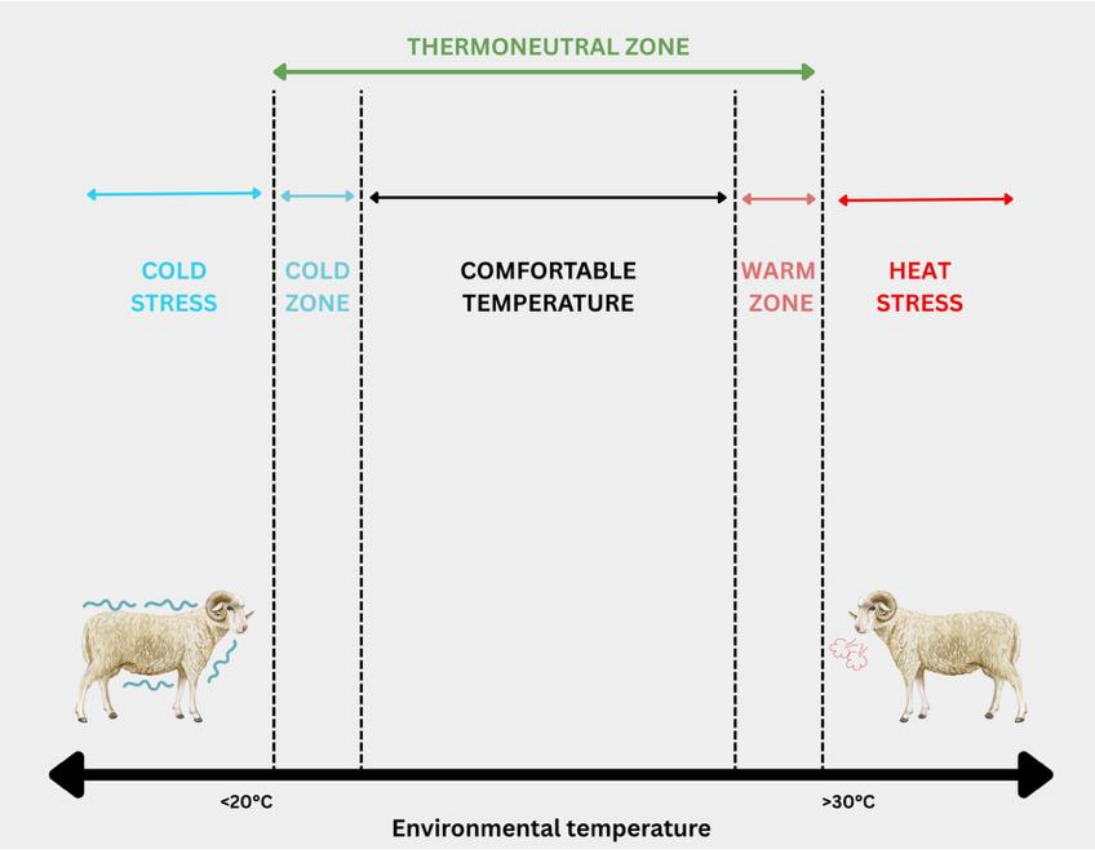

Figure 3. Illustration of sheep's thermoneutral zone (ASTUTI et al., 2022)

Figure 3 illustrates the thermoneutral zone, the HS zone and the cold stress (CS) zone, which are located in the extreme point in both directions. According to DOS

SANTOS et al. (2021), animals' metabolic rates are at a low when they are in the thermal comfort range because they are confined between the lower critical temperature (LCT) and the upper critical temperature (UCT) in the thermoneutral zone. At this moment, the animal's body temperature is in balance with its surroundings as it does not require any energy to maintain a higher internal temperature. Instead, all of its resources are focused on achieving optimal performance, such as production and reproduction. Visible heat exchange methods are adequate here for maintaining thermal equilibrium. If it exceeds the UCT, the animal is under HS. Under such circumstances, animals may attempt to adapt their behaviour by moving to cooler areas, seeking shelter near cooler surfaces, increasing their water consumption, elevating their heart rate, and reducing their food intake. Sheep of various breeds have different ranges for their thermoneutral zones. For example, the ideal thermoneutral zone for a lactating dairy cow is between 5 and 25 °C (BECKER et al., 2020) and as an upper limit, some have determined that 27 °C is optimal for cattle in general (HERBUT et al., 2018). For goats, they typically have a thermoneutrality zone between 25 and 30 °C (LIMA et al., 2022). Sheep with wool coats were observed to produce consistent metabolic heat within the temperature range of 15 to 35 °C, whereas sheep with hair coats performed so within the temperature range of 20°C to 30°C. This differential can be attributed to the innate thermoregulatory capacity of sheep, which allows them to efficiently dissipate excess heat (DE FRANÇA CARVALHO FONSÊCA et al., 2019).

In comparison to other ruminants, sheep generally exhibit superior performance in adverse environments; however, heat stress adversely affects livestock farming in numerous ways, including diminished production efficiency (FAVERDIN et al., 2022; GUPTA et al., 2022), disruption of physiological processes, development, and reproductive functions (FABRIS et al., 2019; VAN WETTERE et al., 2021), among others. Heat stress is harmful as it alters biological molecules, impairs cellular functions, impacts metabolic responses, induces oxidative cellular damage, activates apoptotic and necrotic pathways (SLIMEN et al., 2015). The negative impact of HS in sheep is presented in Figure 4, which further elaborates.

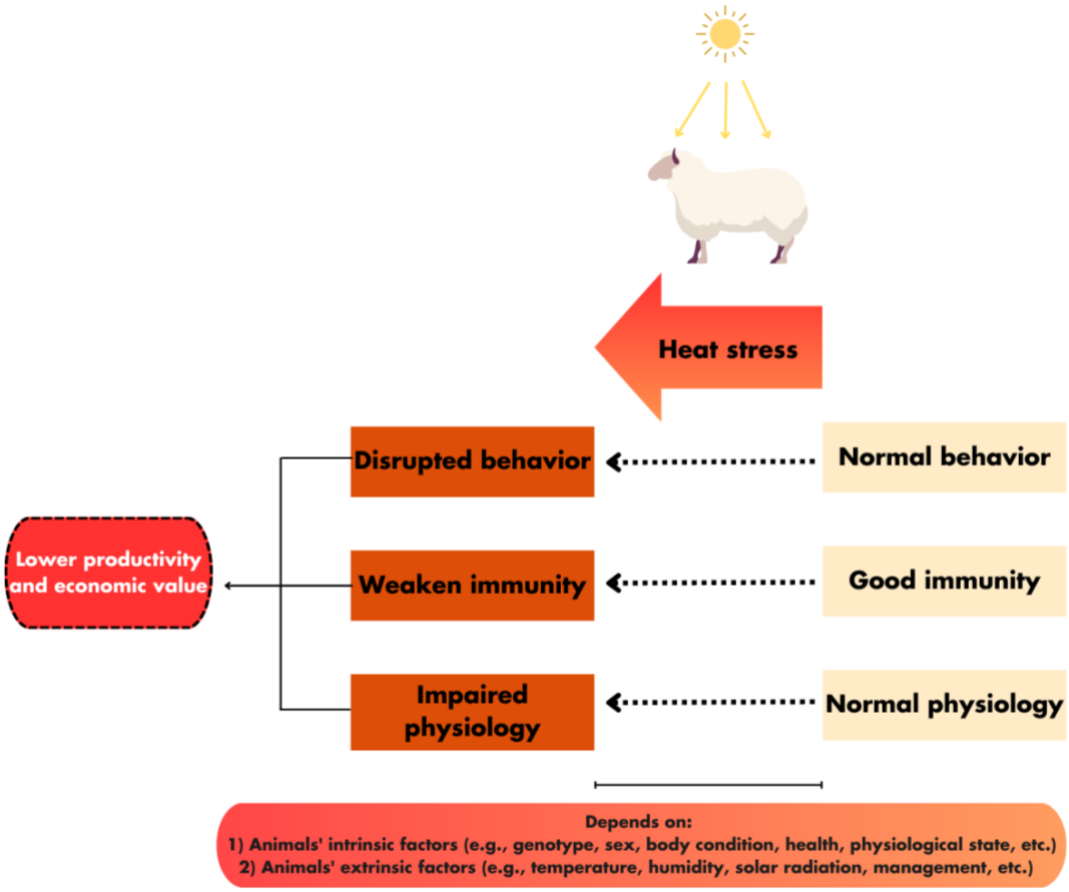

Figure 4. Heat stress deleterious impact to livestock through various ways (ASTUTI et al., 2023)

2.2.1. Physiological and behavioral responses

HS triggers various physiological reactions to maintain homeostasis, including elevated body temperature, accelerated blood circulation and increased respiration rate through panting, and sweating, which helps in the dissipation of heat (ABOUL- NAGA et al., 2021). HS leads to an elevation in skin temperature, which causes the dilation of the capillaries in the skin. This dilation subsequently enhances blood circulation to the skin's surface. It facilitates the dissipation of heat by sweating, promoting the transfer of heat from the surrounding environment to the body's cooler surface through convection (DE et al., 2020). Likely process with the increased respiration rate and panting, which facilitate rapid evaporation cooling (MARCONE et al., 2021).

Heat stress affects ingestive behaviour and can reduce or hinder ruminating process. Heat-stressed sheep exhibited increased ruminating behaviour ($p = 0.011$) compared to non-heat-stressed sheep, but non-heat-stressed sheep had greater panting than heat-stressed sheep ($p = 0.001$). Heat-stressed sheep exhibit prolonged periods of inactivity to

mitigate the rise in body temperature resulting from physical activity (MARCONE et al., 2021).

These processes primarily involve autonomic responses that are activated by the autonomic nervous system (ANS) through the action of catecholamines (adrenaline and noradrenaline). These responses may involve stimulation of energy mobilization from the body's reserves, an acceleration of muscle glycogenolysis, and the inhibition of energy storage, resulting in metabolic alterations (GONZALES-RIVAS et al., 2020). These increased physiological rates during heat stress have been reported in several sheep species, e.g. Corriedale sheep (KITAJIMA et al., 2021), Barki sheep (ABOUL-NAGA et al., 2021) and Malpura sheep (MAURYA et al., 2019).

2.2.2. Production ability and product quality

Lambs exposed to heat showed a decrease in their growth rate and feed efficiency, while their dry matter intake remained unchanged compared to lambs in a thermoneutral environment. HS modified the metabolic state of lambs after food absorption, resulting in a metabolic environment characterized by high levels of insulin. This environment suppressed the breakdown of fat in adipose tissue, promoted the production of lipids, and raised the availability of glucose in cells. Decreased levels of glucose, cholesterol, total protein, urea, potassium, thyroid hormones, erythrocyte and platelet counts, haemoglobin, and hematocrit were observed, while increased levels of serum triglycerides, chlorine, erythrocyte size, and leucocyte count were found. These findings may suggest a compromised immune function (NICOLÁS-LÓPEZ et al., 2021). Studies conducted by SHI et al. (2020) and RATHWA et al. (2017) have shown that HS has a negative impact on lambs' antioxidant status and immunological response. This negative effect is caused by oxidative stress, changes in body temperature, hormonal and behavioural adaptations, changes in circulation, and other complex mechanisms.

Some evidence of body weight loss was found in Dorset cross and Dorper cross sheep (ZHANG et al., 2021) and in Dorper x Katahdin male lamb but no change in feed intake was observed (MACÍAS-CRUZ et al., 2020). The main factor contributing to this issue is the significant reduction in feed consumption during periods of heat stress, which therefore results in a low feed conversion efficiency. Furthermore, the majority of energy use is dedicated to maintaining homeostasis (DE et al., 2023).

HS not only presents symptoms in live animals but also has detrimental effects on the quality of their products, including the production of milk and meat quality post-

slaughter. As the body requires additional energy to maintain homeostasis, livestock, on the other hand, tends to decrease feed intake and increase water consumption in anticipation of increased heat accumulation within the body. This is another mechanism of thermoregulation aimed at reducing heat production in the rumen and consequently lowering the body's internal heat load (HILL and WALL, 2017). Ruminal fluid pH and microbial flora can also become unbalanced due to HS, which impacts digestion and ruminant function (LI et al., 2024a). Therefore, in such circumstances, sheep reduce their anabolic activity and accelerate the breakdown of fat and muscle tissue in order to improve the availability of essential nutrients without impacting their feed consumption (MACÍAS-CRUZ et al., 2020).

HS reduces Hu sheep's growth performance by altering the activities of metabolic enzymes and causing a change in the energy metabolism of the longissimus dorsi muscle, making it more glycolytic and less oxidative. This, in turn, affects the quality of the meat that Hu sheep produce. The heat-stressed Hu sheep showed a change in muscle fibre type towards less oxidative fibres, as indicated by the downregulation of four antioxidant genes in the longissimus dorsi muscle (ZHANG et al., 2023). According to XING et al. (2019) and GREGORY (2010), heat-stressed sheep produce meat that is darker, dryer, and harder, attributed to a high post-mortem final pH (>6.0), which is attained by increased muscle glycogenolysis and anaerobic metabolism. In addition, these hormones are associated with a reduction in the amount of muscle glycogen after slaughtering, leading to inadequate amounts of lactic acid production to lower the final pH below 6.0.

Sheep milk has to contain a significant quantity of fat and protein to ensure the manufacture of excellent cheese, as it is mostly utilized for cheese-making purposes. HS, according to CAROPRESE et al. (2012), can change the composition of milk by lowering fat and protein levels. Elevated ambient temperatures can also lead to a disruption in the mineral composition of plasma, mostly by reducing the levels of sodium, potassium, calcium, and phosphorus, while simultaneously raising chloride concentrations. Milk lipolytic and proteolytic enzyme levels raised as a consequence of an increase in capillary permeability and neutrophil concentrations brought about by exposure to UV radiation at high ambient temperatures. In Lacaune sheep, ewes subjected to HS milk exhibited reduced fat content (−1.7 points) and protein content (−0.86 points). Moreover, HS milk had a higher concentration of somatic cells (+0.23 log points) in comparison to the thermoneutral ewes (MEHABA et al., 2020). In terms of milk production, a study in Sarda ewes by PEANA et al. (2007) found that if the minimum temperature reached 21°C, up

to 15% of milk yield was lost, whereas in Valle del Belice sheep and Italian tropical breeds, 3.9% of milk production was lost when the THI exceeded 23 (FINOCCHIARO et al., 2005).

2.2.3. Reproductive performance

The climate seems to have a major impact on regulating the reproductive patterns of small ruminants. Although the main environmental element that controls the circadian and seasonal rhythms of oestrous and ovulatory activity in sheep is photoperiod, temperature fluctuations can modify the endocrine and molecular mechanisms that regulate the cyclicity of these reproductive events (MARAI et al., 2008). In addition, in the case of animals experiencing heat stress, when a lack of nutrients is identified as a result of an imbalanced energy supply inside the body, heat stress also disturbs the hormonal equilibrium, which is a vital factor in reproduction. During the initial phases of a sheep's development, extreme heat may hinder the normal development of the fetus (CHEN et al., 2021).

GHARIBZADEH et al. (2015) investigated the maturation of ovine oocytes in vitro after a 12-hour heat shock at 41°C. The negative effects of HS on oocyte maturation and the meiotic apparatus of the oocyte could have severe consequences for pre- and post-implantation development. In addition, HS accelerated zona pellucida dissolution, which may be associated with premature cortical granule exocytosis. Exposure of pregnant ewes to warm ambient temperatures throughout mid- and late-gestation leads to a significant decrease in the total number of embryo cells and the size of the placenta (VAN WETTERE et al., 2021). In addition, the incidence of embryo mortality in short-term heat-stressed ewes is 12.7% greater than in thermoneutral ewes, followed by an increase in the number of unfertilized ova (KANDEMIR et al., 2013; ROMO-BARRON et al., 2019).

According to INDU et al. (2015a), HS significantly decreased plasma estradiol and progesterone levels in sheep due to decreased Gonadotrophin-releasing hormone (GnRH) production. Heat stress in sheep decreases oestradiol levels and aromatase activity, adversely affecting the occurrence and duration of oestrus. Furthermore, WAKAYO et al. (2015) noted that lower peripheral gonadotrophin levels may lead to decreased ovarian follicular development, which in turn reduces oestrogen concentrations. For example, in sheep of indigenous Indian breeds that were exposed to HS, the oestrous cycle was found to be disrupted, leading to a shorter and less intense behavioural oestrus (6-8 hours)

(INDU et al., 2015b). Ewes may also experience delayed ovulation and changed ovulatory symptoms and behaviours (SA'AYINZAT et al., 2021). Stress from high temperatures affects when and how follicles mature before an egg develops, and it also causes progesterone levels to rise in the blood during the luteal phase. What this means is that progesterone production is improved (ROMO-BARON et al., 2019).

Also, at 32°C, the correlation between the lambing rate, the number of mating days per week and the fertility of ewes was negative. For every additional day of 32 °C during mating week, the lambing rate and ewe fertility fell by 3.5% and 2.7%, respectively. Heat stress reduces ewe's fertility during mating season; thermoneutral ewes have a 2.4-fold higher chance of becoming pregnant than heat-stressed ewes (KLEEMANN and WALKER, 2005; ROMO-BARON et al., 2019).

In addition to ewes, disruptions in fertility are also found in ram. The semen of rams with higher testicular and scrotal temperatures has a negative correlation with pregnancy rates. This is because the semen quality is lower, there is a higher presence of damaged sperm, and a smaller proportion of sperm that are motile. Increases in testicular temperature caused by the environment can lead to the mortality of germ cells, harm to DNA, and interference with the process of sperm maturation. The sperm may experience damage, which can lead to infertility, hindered development of embryos, genetic diseases in the progeny, growth problems after birth and reduced lifespan of litters (HAMILTON et al., 2018; VAN WETTERE et al., 2021).

In a 6-hour experiment involving Malpura sheep housed in a climatic chamber set at 42 °C with 54% relative humidity, haemoglobin and packed cell volume levels decreased notably as more water circulated through the animals' thermoregulatory system. The heat-stressed rams also exhibited higher plasma cortisol levels and enhanced cholesterol breakdown, which supported increased gluconeogenesis and energy production. Additionally, reduced levels of triiodothyronine (T3), thyroxine (T4), and testosterone were detected, accompanied by alterations in libido, sperm concentration, sperm motility, and amount of semen. A reduction in hypothalamic release of gonadotropin-releasing hormone (GnRH) was thought to be responsible for these alterations (MAURYA et al., 2017; SHAHAT et al., 2020). Furthermore, KASTELIC et al. (2017) and MARAI et al. (2008) also reported results that were comparable, that heat stress reduced the reproduction performance in male and female sheep.

Rembi rams in dry parts of Algeria showed reduced libido all summer long when temperatures hit 39.5 °C (BENIA et al. 2013). Seasonal changes may affect the sperm

quality of Ouled Djellal rams, according to BELKADI's (2017) research, which shows that environmental temperature affects male reproductivity, especially in heat stress situations. Moreover, data from different sheep breeds show that heat stress reduces a number of ejaculatory parameters, including the latency period, the number of mounts for the first ejaculation, the total duration for the second ejaculation, the number of mounts for the second ejaculation, and the reaction time for the first ejaculation (RAHIM & AMIRI, 2023).

2.2.4. Immunity, oxidative stress, and disease occurrence

Sheep become more susceptible to specific diseases as a result of HS's harmful impact on immunity and other biological processes in the body (Figure 5). According to INBARAJ et al. (2016), when an animal recognizes a disturbance to its balance or in a stress condition, its biological response begins, which may be seen as a change in metabolism. An animal's immune system is able to adapt to new threats because, when threatened, the central nervous system communicates with any part of the body in order to mitigate or compensate for the threat. By affecting the balance of two immune system proteins, HS can make animals more susceptible to disease. These proteins, T-helper 1 (Th1) and T-helper 2 (Th2), are responsible for creating proinflammatory and anti-inflammatory responses, respectively (LI et al., 2024a).

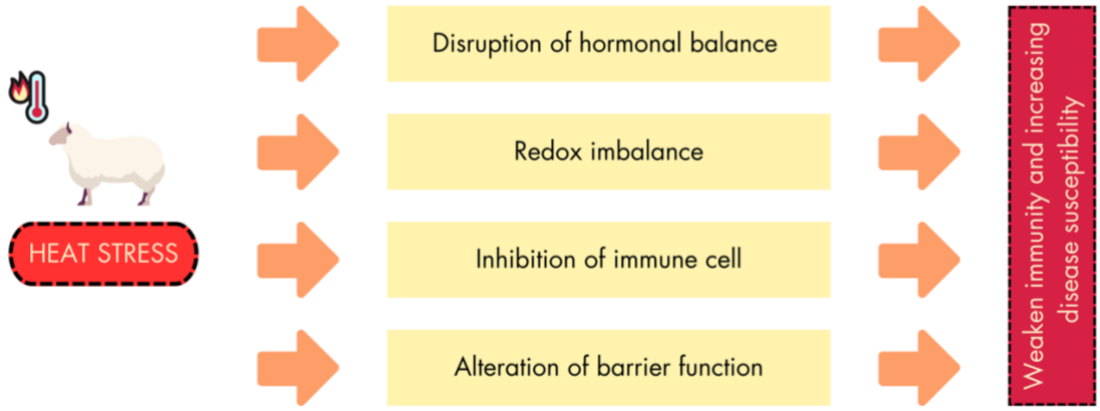

Figure 5. Heat stress weaken immunity and increases disease susceptibility (ASTUTI et al., 2023)

The immune system is composed of two subsystems: the innate and the adaptive. The adaptive immune response can only be developed after exposure to pathogens, although the innate immune system can operate independently (GUPTA et al., 2022). When HS occurs, it disturbs both immune systems. The immune system's initial line of defence against foreign stressors is vulnerable to HS. The production of specific antibodies against foreign proteins or antigens by cells such as natural killer cells, antigen-presenting cells, T- and B-lymphocytes, and others is known as adaptive immunity. This HS modifies immunological responses by activating the HPA and sympathetic-adrenal-medullary (SAM) axes, which regulate the body's reaction to stress (CANTET et al., 2021).

An increase in glucocorticoid levels occurs when the body's HPA axis is activated in response to heat, which causes a surge of hormone releases. Acute stress causes glucocorticoid levels to rise, which act on critical systems to maintain homeostasis (INBARAJ et al., 2016). As soon as the stressor goes away, the body's production of glucocorticoids decreases and eventually returns to normal (DALLMAN & BHATNAGAR, 2011). When stresses are present all the time, however, the HPA continues to secrete glucocorticoid, keeping blood levels high. Because the negative feedback process is impaired, this is made severe (GJERSTAD et al., 2018).

Since HS raises reactive oxygen species (ROS) production and/or decreases antioxidant levels, it causes oxidative stress and an imbalance between oxidants and antioxidants, making animals more susceptible to infections and producing diseases (CHAUHAN et al., 2021). According to ELLAMIE et al. (2020), ruminants' circulatory systems became dysfunctional, and their organs were damaged as a result of prolonged exposure to high temperatures, which redirected blood flow away from vital splanchnic tissues like the liver and gut and towards the skin and lungs. Reduced blood flow to the splanchnic region led to cellular hypoxia, which in turn raised free radical production (both oxygen and nitrogen derived) above cells' antioxidant capabilities. This could damage the intestines through oxidative stress and allow the passage of bacteria and their toxins into the bloodstream through the intestinal lumen. Because it causes oxidative tissue damage and systemic inflammation and affects endocrine functioning, HS may disturb animal metabolism, especially glucose and lipid metabolism (SEO et al., 2024).

Cortisol levels were elevated in heat-stressed ewes; the elevation in cortisol release may have compromised their cellular immune response after receiving an intradermal mitogen injection and their generation of Immunoglobulin G (IgG) after receiving an

antigen injection (CAROPRESE et al., 2012). In the case of dairy sheep, the health of the udder is a crucial factor to take into account. Evidence from bacteriologically positive milk samples taken from ewes subjected to direct solar radiation suggests that HS can reduce the mammary defence capacity, leading to increased bacterial colonisation of sheep udders, by identifying environmental pathogens among the microbiological species. Because of this, udder health is compromised, and milk quality drops (HABIMANA et al., 2023; KARAGEORGOU et al., 2023).

Extensive research has been conducted on the impact of stress on the functioning of the immune system. It has been observed that acute severe stress leads to immune activation, whereas chronic stress results in immune repression (DHABHAR, 2008; CANTET et al., 2021). Cortisol release at periods of acute stress serves as an immune system stimulus; nevertheless, the release of cortisol during chronic stress has been associated with a decrease in immune function, rendering an animal more susceptible to diseases and immune-related issues. In addition, different cytokines indicate compromised immunological responses, and the impact of HS stress on producing glucocorticoids varies accordingly (SOPHIA et al., 2016a; BAGATH et al., 2019). Some evidence suggests that ruminants' immune responses are related to their responses to other forms of environmental stress. For example, ruminants have been found to experience increased blood cortisol levels (BAGATH et al., 2019), changes in gene expression that are involved in immunity (SRIKANTH et al., 2017), or a reduced response to mitogen stimulation leads to a decline in the number of viable cells among bovine blood mononuclear cells (BMCs) (LACETERA et al., 2006), or increasing the pathogen load in dairy animal, which lead to higher occurrence of mastitis. It is also reduced the IgG circulation by 27% and disturbed inflammatory cytokines circulation, the immune-related pathways; Toll-like receptor, T- and B-cell receptors signaling pathways are upregulated in the blood as the response of high temperature that negatively impact immune function (DAHL et al., 2020).

2.3. Adaptation to heat stress

Adaptation reactions refer to adjustments in ongoing physiological processes that enable an animal to adjust to stressful stimuli while minimizing disturbances to homeostasis (NEJAD and SUNG, 2017). BERIHULAY et al. (2019) define adaptation as the capacity for tolerance necessary for survival and reproduction under harsh living conditions. Indigenous livestock breeds are characterised as the most resilient, capable of

thriving and producing in difficult environments due to their physiological and genetic adaptations. Indigenous or locally adapted breeds exhibit better resistance to HS than international modern breeds. The international breeds in question are generally heavier, shorter and possess larger bones compared to the indigenous/local breeds, with taller animals seen more efficient at heat dissipation than those with short, muscular bodies. Despite lower production of indigenous breeds capacity compared to modern and crossbreeds, their output levels remain generally consistent under experimental situations when high-producing animals fail. Smaller indigenous breeds are better able to maintain their reproductive potential in the face of severe heat stress, water scarcity and reduced pasture availability than larger international animals, whose higher energy demands may cause them to suffer reproductive deficiencies. Thus, breed definition also mirrors breed history, therefore adaptation often aligns with their natural history of territorial occupation (SEJIAN et al., 2018; MCMANUS et al., 2020).

The primary mechanisms of heat stress adaptation in sheep include changes in morphology, behaviour, physiology, biochemistry, metabolism and molecular and cellular processes. Animals are able to maintain a constant internal temperature through a process known as thermoregulation, which involves a variety of physiological, biochemical, and physical mechanisms (AL-DAWOOD, 2017).

2.3.1. Physiological adaptation

Various physiological responses are triggered in order to maintain an equilibrium core temperature in response to heat stress. Vasodilation, panting and sweating are physiological mechanisms employed by animals to enhance heat dissipation. The existence of a wool coat makes respiratory evaporation significantly more essential than sweating in wool sheep. On the other hand, sheep can also generate unconscious perspiration, which is a kind of water diffusion through the skin that helps to remove heat from the body's surface. These mechanisms are manifested through behaviours such as rapid respiration and increased heart rate. They also lead to an elevation in rectal temperature and in-vivo oxidative metabolism. Additionally, it causes disruption in water and electrolyte balance, resulting in increased water intake and reduced food intake (LI et al., 2018; DAHL et al., 2020; MCMANUS et al., 2020; KITAJIMA et al., 2021). The increase in water consumption is a result of the loss of body fluids, which can be intensified by exposure to high temperatures. Conversely, the decline in food consumption is primarily a strategy to minimize heat generation during the process of

digesting feed (NEJAD and SUNG, 2017). As an example of behavioural adaptation, DE et al. (2015) mentioned that grazing ruminants, when subjected to HS, lie down during the day to minimise their movement. According to NEJAD and SUNG (2017), sheep that were dehydrated spent more time sitting and less time standing, lending validity to this argument.

An elevated heart rate is one of the most obvious physiological effects of HS since it changes blood flow in sheep (STOCKMAN, 2006). One of the first things that happens when your body experiences heat stress is vasodilation, which helps keep your core temperature stable. The decrease in sympathetic brain activity, which controls the tone of blood vessels, initiates this reaction. As the heat load increases, latent heat dissipation begins to take place. In addition to regulating the activity of sweat glands, cholinergic sympathetic activity also helps dissipate heat by acting as a local vasodilator (KITAJIMA et al., 2021).

2.3.2. Cellular adaptation

Various cellular behaviours must undergo adjustments in response to environmental stresses. The impact of heat stress on animal production can be explained by changes in cellular function, such as decreased DNA replication, transcription and translation; protein denaturation, misaggregation and degradation; metabolic changes that result in a net decrease in cellular ATP; and changes related to the membrane (HYDER et al., 2017).

Thermal stress can modify normal biological responses, as well as impact metabolic reactions and cell membrane activities. It can also cause oxidative damage to cells, trigger necrosis and apoptosis pathways, and eventually result in cell death. Livestock depend significantly on the HPA axis to regulate their hormonal reactions to HS. The heat stress induces a physiological adaptation that alters how the body uses energy from the body storage. This leads to an excessive production of glucocorticoid, the primary hormone that helps animals cope with stress caused by heat. The production of glucocorticoid increases in response to the activation of the HPA axis (FULDA et al., 2010). Glucocorticoids increase the rate of myofibrillar protein breakdown in skeletal muscle, mediated by the Ca^{2+} dependent, ubiquitin-proteasome and the autophagy-lysosome system, and thereby improve heat loss through vasodilation. Additionally, the anabolic regulators insulin and insulin-like growth factor-1 (IGF single bond I) are inhibited by glucocorticoids, leading to even more severe muscle mass loss. Glucocorticoids increase the expression of fatty acid synthase in hepatocytes and adipocytes, which in turn

increases lipogenesis (from glucose and glucose precursors) and lipolysis (mediated by increased hydrolysis of circulating triglycerides by enhanced lipoprotein lipase activity) (GONZALEZ-RIVAS et al., 2020).

Prolonged exposure to high temperatures induces various physiological reactions and triggers the activation of specific genes, leading to changes in protein synthesis through transcription and translation processes. Among these alterations are the following: immunological and endocrine function suppression, amino acid changes in the blood, decreased bioavailability of cellular energy, increased expression of *HSP* mRNA, upregulation of inflammatory genes (*NF-B* and *TNF*), *PMEL* and *MC1R*, and decreased antioxidant enzyme levels (HOOPER et al., 2018).

When animals are under stress, their neuroendocrine systems go into hyperactivity, causing them to secrete tropic hormones and activate many hormonal pathways, all of which aid in adaptive and behavioural responses. Hormonal shifts are characterised by an increase in catabolic and a reduction in anabolic hormones. Adrenocorticotropic hormone (ACTH), thyrotrophic hormone (TSH), somatotropic or growth hormone (GH), follicle-stimulating hormone (FSH), luteinizing hormone (LH) and prolactin (PRL) are the most prominent players in this group. Plasma concentrations of these hormones may serve as possible indications of physiological alterations in heat-exposed animals (BERNABUCCI et al., 2010; MCMANUS et al., 2020). Triiodothyronine (T3) and thyroxine (T4) concentrations in the blood and tissues are known to decline during heat stress. This is probably because the hypothalamo-pituitary-thyroid axis is directly impacted by heat stress, which reduces thyrotropin-releasing hormone production and limits basal metabolism (PRAGNA et al., 2017).

The longer the duration of HS, the less active the antioxidant enzymes may be. An essential enzyme found in many different kinds of tissues and animals, superoxide dismutase (SOD) neutralises the harmful effects of the superoxide anion radical and its product on cells. The concentration of reactive oxygen metabolites (ROM) and SOD activity both rise in response to HS. Heat stressed body either increase the formation of free radicals originating from oxygen or reduced antioxidant defence, both of which contribute to oxidative stress (DI TRANA et al., 2007; SHI et al., 2020).

2.3.3. Morphological adaptation; skin type and hair color of sheep

The morphological feature of animals determines their adaptability to nature. According to GEBREMEDHIN et al. (2008), the adaption of HS is influenced by crucial

elements such as skin type and colour. The sweat gland density, function, morphology and the density, length and color of hair coat determine the efficiency of heat dissipation from the skin surface. When tackling HS, it is crucial to take into account the skin and hair as they serve as the outermost protective barrier of an organism's body, shielding it from exposure to thermal stressors. Sheep exposed to high temperatures, as explained by MACÍAS-CRUZ et al. (2018), engage in evaporative thermoregulation mechanisms. Unlike other ruminants, where sweating is required to avoid hyperthermia, sheep have a unique ability to regulate their body temperature by altering their breathing rate, allowing them to remove 60 to 90% of heat load. In contrast, sweating only accounts for less than 10% of heat dissipation in sheep. The adaptive capacity of animals is affected by the external attributes of their coat, such as coat color and physical hair traits. The characteristics of an animal's coat are significant traits that can be utilized as a criterion for selecting animals. Furthermore, these attributes are associated with the animals' ability to regulate their body temperature and maintain homeostasis.

The number of apocrine glands is strongly related to hair density. Denser coats might make it more difficult for latent heat to evaporate through the skin. In sheep, secondary follicles create wool after primary follicles form hairs with heterotypic strands. Wool is therefore not linked to a sweat gland (DO PRADO PAIM et al., 2013). Wool not only provides insulation but also reduces perspiration by making it harder for water to evaporate from the body. Thermoregulatory abilities are impaired in woolly sheep. Thicker wool contains characteristics that lessen thermoregulation via convection, even though it has a lesser insulating value. Even with thinner wool, the inside of the fleece has air stability, resulting in less heat loss by convection (MCMANUS et al., 2020).

According to YIN et al. (2019), coat color develops through two pigments produced by melanocytes, which are skin cells in the epidermis. These cells generate two types of melanin: pheomelanin (yellow to reddish) and eumelanin (black to brown). The differences in skin and hair color depend on the amount and balance of these pigments. Both genetics and environmental (e.g., sunlight, nutrition and hormonal changes) influences affect how melanocytes produce and store melanin.

The adaptation of sheep to hot settings is influenced by both their hair type and color. Various research has been undertaken to verify the hypothesis that light colors have a higher capacity to reflect sunlight compared to dark colors, specifically in relation to the coat color of animals. OKORUWA (2015) found that sheep with a black coat color, low coat depth and short hair length are at a greater risk of HS because their dark

pigmentation, low coat depth and short hair cannot effectively protect them from solar radiation, particularly direct sunshine. MCMANUS (2011) discovered a similar finding in Brazilian Santa Ines, Bargamasca and crossbred animals. The white coat is superior because it enhances epidermal protection by absorbing short-wave ultraviolet rays, which is crucial in light of epidermal depigmentation, making animals vulnerable to erythema, burns, and neoplasms (LEITE et al., 2020).

A different finding was made in Indian sheep breeds. A comparison of haemato-physio-biochemical traits of Chokla, Marga and Marwari sheep with different coat colors revealed no significant differences; all of these sheep are equally adaptable under hot conditions (SINGH et al., 2016). Similarly, LEITE et al. (2018) observed Morada Nova ewes with varying coat colors. There was no color difference in the animals' rectal temperatures, and all animals could maintain homoeothermic settings; however, each group activated different heat loss pathways. The most significant distinction was observed in the completely white coat, which exhibited modified thermoregulatory responses along with the highest rate of perspiration. The white-coated Naemi breed of Saudi sheep has a noticeably higher rectal temperature than the black-coated Najdi type, according to studies conducted by AL-HAIDARY et al. (2021). Meanwhile, neither breed differed significantly from the other with respect to skin temperature, packed cell volume, or plasma albumin levels. In contrast to white-coated animals, black-coated animals had much greater plasma globulin and total protein levels. Researchers found that black-coated animals had a far higher heat tolerance coefficient than white-coated animals. According to the results, sheep grazing in a hot desert environment were not more heat tolerant because of their lighter coat colour.

2.4. The genetics of thermotolerance

As their environments change, organisms undergo a continual process of evolution in which they alter their gene combinations. A crucial element of adaptation is the genetic capacity of an organism to endure harsh environments. Genetic diversity is key for climate change adaptation, as it allows for genetic selection of heat-tolerant traits that persist across generations. Combined with better management, the genetic gain is permanent, therefore it will last across generations, making this a sustainable solution (OSEI-AMPONSAH et al., 2019).

However, it will not be an easy task, as BERIHULAY et al. (2019) stated that the development of heat tolerance in livestock involves an intricate network of genes that

coordinate across multiple systems, with the objective of mitigating the consequences of heat stress at the cellular level. Moreover, at some point, the adaptability has genetic antagonism with production ability due to the opposite direction in energy utilization (OSEI-AMPONSAH et al., 2019; WORKU et al., 2023).

Genome and genomic studies are aiding in the investigation of thermo-tolerance genes and genomic regions that are crucial for regulating body temperature in sheep by study the interactions between different breeds that share geographic regions, thereby effectively capturing the influence of several mutations that lead to variances in heat tolerance (ABOUL-NAGA et al., 2022). Heat stress induces a wide spectrum of cellular and molecular reactions that can be characterized via molecular biotechnology methods, such as gene expression analysis, enabling the identification of essential biological responses to heat stress. These genetic markers are crucial in understanding the genetic variation in heat-stress adaptation genes and their intricate networks, which further be incorporated in genomics selection for climate change adaptation. Moreover, alongside the increasing trend in the integration of omics studies, discovering the HS control network that can detect a panel of markers that can be used in the selection of not only heat-tolerant animals, but also with higher productivity (SEJIAN et al., 2018; WORKU et al., 2023).

A number of potential genes linked to heat tolerance in sheep have been identified through diverse investigations, as indicated in Table 1.

Table 1. Some of heat tolerance candidate genes in sheep

Candidate genes	Function	Ruminant species	Reference
70kDA heat shock proteins (*HSP70*), 90kDA heat shock proteins (*HSP90*)	Heat stress response	Sheep	YOUNIS (2020)
Superoxide dismutase type 1 (*SOD1*), Superoxide dismutase type 2 (*SOD2*)	Antioxidant capacity	Sheep	LI et al. (2019a)
F-box protein 11 (*FBX011*), polyhomeotic homolog 3 (*PHC3*), Thyroid stimulating	Cellular adaptation to heat stress	Sheep	LUNA-NEVÁREZ et al. (2021)

Candidate genes	Function	Ruminant species	Reference
hormone receptor (*TSHR*), Signal transducer and activator of transcription (*STAT1*)			
Myosin VA (*MYO5A*), Protein kinase CGMP-dependent 1 (*PRKG1*), Glutathione S-transferase C-terminal domain containing (*GSTCD*), Reticulon 1 (*RTN1*)	Pigmentation, smooth muscle contraction, lung function, endoplasmic reticulum stress	Sheep	ABOUL-NAGA et al. (2022)
Interleukin 2 (*IL2*) and Interleukin 6 (*IL6*)	Cellular homeostasis	Sheep	RAWASH et al. (2022)
Glucocorticoid modulatory element binding protein 2 (*GMEB2*) and Inositol polyphosphate-5-phosphatase B (*INPP5B*)	Central control in heat stress signal transduction	Sheep	HAIRE et al. (2022)
Interleukin 10 receptor, beta subunit (*IL10RB*) and Interleukin-23 subunit alpha (*IL33A*)	Immune response	Goat	ONZIMA et al. (2018)
Macrophage stimulating 1 (*MST1*), Phosphoenolpyruvate carboxykinase 1 (*PCK*), and Secreted Frizzled Related Protein 1 (*x*)	Hypoxic adaptation	Goat	WAINEINA et al. (2020)
Glutamic-oxaloacetic transaminase (*GOT1*) and Cadherin 2 (*NCAD*)	Stress response, cell differentiation, and	Cattle	LUNA-AZUARA et al. (2024)

Candidate genes	Function	Ruminant species	Reference
	homeostatic process		
Heat shock protein family A member 13 (*HSPA13*), DnaJ heat shock protein family member C18 (*DNAJC18*), and DnaJ heat shock protein family member (*C8DNAJC8*)	Molecular chaperoning	Cattle	AYALEW et al. (2024)

Several genes, including Heat stress proteins (*HSP*s) (CHENG et al., 2018; UMAR et al., 2021), Interleukins (*IL*s) (TSUGAMI et al., 2021), Toll-like receptors (*TLR*s) (JU et al., 2014; BHARATI et al., 2016), and countless others have been found to play important roles in thermal adaptation by acting as molecular chaperones, suppressing protein synthesis and facilitating transport proteins during HS. The HSPs have been the subject of many of research into their regulation, cellular localisation and physiological roles. There is a vast network of proteins that work together to maintain proteostasis and HSPs are just one of them. The fact that HSPs are synthesised in response to various stresses indicates that these "housekeeping" proteins are vital to the biology and physiology of cells that are under stress. As a biomarker, HSPs are indisputable because they play a chaperone role in the folding, unfolding, and refolding of stress-denatured proteins; the cell's final reaction to HS is elevated amounts of HSPs and the expression of thermotolerant genes. The majority of the HSP research has focused on the *HSP60*, 70, and 90 families. The stress response gene *HSP70* has been the subject of extensive research in animals because of its abundance, sensitivity, and high degree of conservation. There is substantial evidence that it correlates with animals' thermotolerance as well (YOUNIS et al., 2020; FANG et al., 2021).

Exposure to heat shock can alter the regulation of immune-related genes, either boosting or suppressing immune cell activity, while also interfering with cellular processes associated with HSPs (RASHMOL et al., 2019). According to MALLIKARJUNAPPA et al. (2020), *IL10* is secreted by dendritic cells, macrophages, regulatory T cells, and certain epithelial cells, and it plays a significant role in regulating inflammation. Evidence suggests that *IL10* makes ovines more susceptible to a number

of diseases, such as the Peste des petits ruminants virus (PPRV), bluetongue virus (BTV), and *Haemonchus contortus* infection. Toll-like receptors (TLRs) are genes involved in immunity that are expressed by many different kinds of cells, such as those on the mucosal surface and immune cells in tissues (CRUZ-TAMAYO et al., 2021). The Toll-like Receptors (*TLRs*) are a family of PRRs that have been around for a long time and can detect a wide variety of microbes' pathogen-associated molecular patterns (PAMPs) (JANEWAY AND MEDZHITOV, 2002). According to BENAVIDES et al. (2016) and GARCÍA-MARTÍNEZ et al. (2022), these factors have an essential role in the host's defence against parasite infections in the gastrointestinal tract and classical scrapie in sheep.

2.5. Molecular genetics approach to understanding heat-stress adaptation in sheep

2.5.1. Polymorphism study in thermotolerance

The study of genetic polymorphism is important for comprehending the architecture of population diversity and species detection, and it holds practical importance for formulating strategies targeting specific characteristics, such as heat resistance. An ability of population to adapt to natural selection is enhanced when its genetic variety is high, since this diversity most likely underpins phenotypic variance (JAIN et al., 2022). SNPs are biallelic changes at single base positions in DNA sequences that are prevalent in genomes and are considered to be the most valuable genetic markers for genetic mapping and association studies. They are irregularly distributed across the genome, occurring in both coding and non-coding areas, and a substantial quantity of SNPs can be genotyped in a single assay (CORTES et al., 2022). SNPs are typically less prevalent in coding regions of the genome than in noncoding regions, which can influence transcription rates, resulting in modifications in the expression of related proteins. Exonic SNPs within coding regions can be categorized into two types: non-synonymous SNPs, which alter the amino acid sequence of protein products, and synonymous SNPs, which do not affect the primary sequence of the products (LIAO and LEE, 2010).

Up until now, there are some polymorphism studies related to environmental adaptation in various sheep breeds, e.g., SINGH et al. (2017) with 13 SNPs located in *HSP90* and *HSP70* genes and their correlation with thermotolerance characteristics in Chokla, Magra, Marwari and Madras Red sheep breeds, and CASTILLO-SALAS et al. (2023) with three SNPs in *PAM, STAT1,* and *FBXO11* genes in Pelibuey ewes. On an advance level, the application of SNPs high density array has been applied in

understanding the adaptation of Creole cattle in a tropical environment using Illumina bovine SNP50 array (PITT et al., 2019), various Mediterranean cattle in Mediterranean climate subtype with Illumina bovine SNP50 array (FLORI et al., 2019), high altitude adaptation in Ethiopian sheep populations with Ovine Infinium HD array (EDEA et al., 2019), Chinese sheep adaptation to extreme environment with 600K high density SNP (ABIED et al., 2020), and local adaptation of Mediterranean Chios dairy sheep using OvineSNP50 Genotyping BeadChip (TSARTSIANIDOU et al., 2021)

In the KASP-PCR method, which stands for Kompetitive Allele Specific Polymerase Chain Reaction, signals are generated using fluorescence resonance energy transfer (FRET). To detect allele-specific amplification for a single bi-allelic SNP, two fluorescent cassettes are used (AM and HEX labelled) (SUO et al., 2020). Upon the presence of the target allele, the corresponding primer binds and amplifies the DNA, incorporating the fluorescent tag. Subsequent to PCR, a plate reader quantifies fluorescence to ascertain the genotype: homozygous samples exhibit a singular colour, whereas heterozygous ones display a combination. The assay performs effectively when data exhibit distinct clusters with great precision. It can be conducted on 96, 384, or 1536-well plates, facilitating the simultaneous processing of several samples (DIPTA et al., 2024). When it comes to biallelic characterisation of SNPs and insertions/deletions at specific loci, this method is quick, cheap, and efficient (ALVAREZ-FERNANDEZ et al., 2021).

A common reverse primer and allele-specific primers amplify the target region in the first round of PCR. The allele-specific primer is built into the template, but the fluor-labelled oligos are unable to glow since they are still bound to the quencher-labelled complementary oligos. During PCR, a fluorescent signal is generated when an oligo with a fluorescence label that corresponds to the amplified allele is integrated into the template and released from its quencher-labelled complement (HE et al., 2014). Various livestock genomics studies have utilized the KASP method for marker-assisted selection; a study of 48 SNPs in 40 genes related to milk production and composition in caprines (KUSZA et al., 2018); ZHANG et al. (2020) identified eight prevalent genetic abnormalities in Holstein cattle, affecting three SNPs and three mutations involving insertions and deletions; 6 SNPs related to milk production in Holstein x Black Pied cattle (MODOROV et al., 2022), and 89 SNPs related to clinical mastitis in Romanian cattle (ILIE et al., 2023).

2.5.2. Gene expression and transcriptomics study in thermotolerance

During HS, transcriptomics can be employed to detect changes in gene expression that are particular to certain tissues. As a result of environmental changes, cells can modify the kind and amount of gene expression, which gives insight into biological reactions at particular points in time (SINGH et al., 2018).

Transcriptomics provides insight into the genetic code, how genes are regulated, the roles played by gene products, and the genome dynamic in a given cell type or tissue under a given set of physiological conditions or developmental stage, all of which contribute to a better understanding of the regulatory network of biological processes in living organisms (DONG & CHEN, 2013). Because it connects genotype to cellular and organismal physiology and, maybe, adaptive phenotypes, the adaption process to fast changing environments can be better understood through the adaptability of gene expression (JOSEPHS, 2020).

Gene expression can be influenced by both genetic and environmental factors. The transcriptome has grown into a powerful resource for studying complex biological processes, such as adaptation, and for determining the nature of the relationship between genotype and phenotype. The expression levels of individual genes and their products can be detected and quantified using a variety of methods, e.g., Northern blot, SAGE, real-time PCR, Western blot and ELISA (CASSAR-MALEK et al., 2008; SHASHANK et al., 2024).

To measure gene expression, one reliable method is quantitative real-time polymerase chain reaction (qRT-PCR), a quick, specific and very sensitive method. The rapid polymerase chain reaction allows for accurate gene expression measurement, pathogen identification and genotyping due to its modest sample volume requirements, multiplexing capacity, and large dynamic range. Also, it utilises a significantly smaller RNA template, can achieve relatively high throughput with the right equipment, has lower coefficients of variation than other gene expression analysis methods, and can discriminate between messenger RNAs (mRNAs) with almost similar sequences. The need for investing in expensive equipment and reagents is the primary drawback of qRT-PCR. Given its extreme sensitivity, it is vital to have a thorough understanding of normalization techniques and solid experimental design in order to obtain reliable information (WONG and MEDRANO, 2005).

According to GINZINGER (2002), qRT-PCR entails three steps: i) RNA to cDNA conversion using reverse transcriptase (RT), (ii) cDNA amplification using PCR, and (iii)

real-time detection and quantification of amplification products (MELING et al., 2018). Several studies have used this method to understand adaptation in sheep. For example, SHI et al. (2020) evaluated the immune system gene expressions in lambs under heat stress. RAWASH et al. (2022) compared the expression of *HSP70, IL2, IL6,* and *IL12* genes in winter and summer Barki sheep. CHEN et al. (2023b) used heat stress treatment in vitro on liver cells and preadipocytes from Hu sheep to explain the molecular mechanism of m6A methylation modification.

Another established method for transcriptome profiling that makes use of deep sequencing technology is RNA sequencing (RNA-Seq). In the course of studying how a gene works in the body, it not only provides a detailed image of the transcriptome but also shows how the molecular structure works. Gene expression in different species, organs, and contexts can be studied using RNA-Seq. According to ZHAO et al. (2017) and SUDHAGAR et al. (2018), it requires thorough preparation of the raw data in order to remove unwanted variance and misleading noise. Although the price of RNA-seq has dropped significantly in recent years, it is still prohibitive for even more extensive use due to issues with data processing and interpretation (HOU et al., 2015; EVARAERT et al., 2017).

Differentially expressed genes may provide evidence that alterations in transcriptome regulation have a role in adaptation, as in order to quickly adjust to the new environment, transcriptional regulation facilitates rapid cellular adaptation (LIU et al., 2015). Animals under HS reactions are based on multiple biochemical pathways, some of which might influence metabolite composition directly. Transcriptomic investigations of temperature adaptation are best conducted in blood because of its central role in homeostasis. KISHORE et al. (2013) states that peripheral blood mononuclear cells (PBMC) are a good biological model for researching the stress response in livestock and provide a reliable stress parameters that can be easily measured (N:L or H:L ratio; DAVIS et al., 2008). PPBMC are a popular cell model because they are representative of the physiological state of the animal as a whole and are easy to extract and cultivate (FANG et al., 2021).

The amount of reads that match a reference transcriptome or genome is what defines RNA-Seq expression according to DE LAS HERAS-SALDANA et al. (2016). Finding novel genes, isoforms, transcripts, and short noncoding RNAs is the primary advantage of RNA-Seq, which is not limited to the array probes. Making a library is an essential first step in an RNA-Seq work. Following RNA extraction, size/type enrichment, and

fragmentation, complementary DNA (cDNA) will be synthesized using random hexamer primers. Following this, the cDNA undergoes a quality control procedure, preparing the cDNA library for sequencing. The next step is to amplify the cDNA to create clusters of double-stranded DNA. This amplification process detects, records, and converts nucleotide additions into base calls. In amplification, the number of cycles determines the read length, whereas the number of clusters determines the read quantity (depth of sequencing). Upon completion of amplification, the raw data, which consists of brief reads, is often exported as FASTQ files. One of the most advanced tools for investigating the adaptation mechanism in livestock recently has been RNA-Seq research. Several studies have been carried out to gain a better understanding of the differences in gene expression in livestock under different environmental stressors. For example, SRIKANTH et al. (2017) examined gene expression in hyperthermic Holstein dairy cows; MORENIKEJI et al. (2020) compared thermoregulation gene expression in tropical and temperate-adapted cattle; HAIRE et al. (2022) examined transcriptomes in Turpan Black sheep and three Kazakh sheep during the summer; and LU et al. (2022) examined hypoxia adaptation in Tibetan sheep.

2.6. Different sheep breed used in the present dissertation

2.6.1. Breeds from Hungary

Suffolk. At the end of the 18th century, local Norfolk ewes were crossbred with Southdown rams, resulting in the Suffolk breed, which has been acknowledged as a distinct breed since 1810. In the 19th century, it disseminated globally, particularly in France, Germany, the United States, Australia, and New Zealand. It is one of the most prevalent and popular varieties of meat globally. It is distinguished by superior maternal characteristics, substantial milk yield, and elevated reproductive rate. It is among the breeds with superior grazing capabilities, however it is also highly adapted to confined housing. In Hungary, the objective of raising this breed is to sustain and enhance desirable maternal traits, elevated reproductive performance, superior meat conformation, and high weight gain (Source: https://mjksz.com).

Bábolna Tetra. The breed was developed at the Bábolna State Farm utilising many rapid breeds, including the Finnish Landrace and Romanov. It is a breed that reproduces continuously throughout the year. The elevated reproduction rate necessitates specialised lambing techniques. The breeding of the breed in Hungary aims to enhance reproduction and lamb yield while preventing inbreeding. Ewes that have produced three consecutive

lambs should be culled to reach and sustain the requisite population size for the selection of breed individuals (Source: https://mjksz.com).

Ile de France. In 1824, this breed was developed to create a productive hybrid of domestic Rambouillet ewes and English Leicester rams, previously referred to as Dishleys. The breed, originally named Dishley Merino, was registered in 1922, the same year it was renamed Ile de France. It was initially developed in Hungary during the 1950s to enhance the attributes of the Hungarian merino. The introduction of the half-breed merino did not result in any issues regarding its domestic distribution. The primary breeding objective in Hungary for this breed is to preserve and enhance its superior maternal traits, early maturity, aseasonality, propensity for twinning, exceptional weight increase, and outstanding meat conformation (Source: https://mjksz.com).

Hungarian Tsigai. Hungarian indigenous Tsigai sheep are an old breed with roots in Asia Minor, which is now Turkey. The second half of the 17th century marked the introduction of this breed to Hungary. In Hungary, throughout the last two centuries, the Tsigai breed has consistently maintained a constant portion of the sheep population, despite varying ratios (1-10%) (GÁSPÁRDY et al. 2006; KUSZA et al., 2010, 2011, 2015).

Hortobágyi Racka. The Hortobágyi Racka (*Ovis aries strepsiceros hungaricus*), a unique Hungarian national breed famous for its morphological characteristics of spiral V-shape horns is often used in crossbreeding because of its resilience and adaptability. This breed presents two predominant coat color phenotypes; uniformly white and uniformly black (CIANI et al., 2020; RAMIREZ et al., 2025).

Hungarian Merino. The Hungarian Merino sheep breed originated from Spanish Merinos introduced to Hungary in 1774 and then evolved through successive crossbreeding with Rambouillet, German Mutton Merino, Russian Merino, Merino Precoce, and other breeds emphasising meat and wool, including Kent, Corriedale, Australian Merino, and Booroola Merino. The breed has been selected developed for meat, wool quality, and reproductive efficiency. Officially acknowledged with a herd book in 1993, the Hungarian Merino is now proficiently adapted to poor pastures and severe, arid, and hot climates, making it a sustainable choice for production in ecologically harshly areas (LOUKOVITIS et al., 2022).

Hungarian Awassi. The Awassi is a fat-tailed sheep, common and significant in the Middle East, mostly for meat and milk production. The unimproved Awassi is a resilient, strong, medium-sized sheep utilised for milk and meat production. The

enhanced dairy variety is larger and more sophisticated than usual Awassi. The first Awassi flock in the temperate zone of Europe was introduced in Yugoslavia. The Awassi flock in Hungary was created through the importation of Awassi ewes in the late 1900s and by crossbreeding Hungarian Merino ewes with Awassi rams, followed by systematic backcrossing with Awassi rams (IZZÓ et al., 2005; BARANYI et al., 2010).

White Dorper. By crossing the Black-headed Persian with the Dorset Horn, the robust South African composite breed Dorper developed in the 1930s. The original intention of the Dorper breeders was to create a sheep breed that could be mass-produced in dry and semi-arid regions for the purpose of making slaughter lambs. Two separate selection procedures resulted in the creation of two Dorper variants: Dorper and White Dorper. Dorper differs from White Dorper in that it is covered in white, but it has a black head and neck (WANJALA et al., 2023). In 2008, the Dorper breed was brought to Hungary from France by Debrecen University with the goal of producing healthy, rapid-growth lambs (BUDAI et al., 2013).

2.6.2. Breeds from Bosnia & Herzegovina

Pramenka. The indigenous Pramenka sheep breed accounts for the vast majority of Bosnia and Herzegovina's sheep. Dub, Kupres, and Privor are the three most major Pramenka strains, and their distribution varies across the country. This study used Dub Pramenka, a place where habitations are connected to Vlašić Mountain. Sheep in mountain regions are typically bred using extensive husbandry practices, such as living on expansive meadows without added feed (VAŽIĆ et al., 2017).

2.6.3. Breeds from Morocco

Béni Guil. The Beni-Guil sheep breed (Also known as "Daghma" and "Hamra"), is still the most popular in the Orient due to its unique characteristics. This breed is native to the area and has spread widely in Morocco due to its adaptability, prolificacy, and performance in fattening and industrial crossbreeding. Their meat is the most important product, but they are mostly multipurpose animals with thin tails. Characteristics of the Béni Guil breed include a square build, a short neck, a brown complexion, and white coat (BOUJENANE & PETIT, 2016; GAOUAR et al., 2016; BELHAJ et al., 2021).

D’man. The D'man breed, often recognised as the most prolific sheep, formerly lived in the southern Algerian Wadi Saorua Valley and the oasis regions of southern Morocco. They are polled (BELHAJ et al., 2021). Some members of the D'man breed

have brown or white fleece in addition to the typical black, but there is also animals with all three colours. They have a thin skull and delicate bone structure. A long tail typically droops over the backs of the hind legs. D'man is valued for its remarkable reproductive abilities (BOUJENANE & PETIT, 2016).

Timahdite. A thin-tailed, multi-purpose breed, the Timadhite is highly valued for its meat. A Timahdite's coat and legs are white, while their face is brown. The Timahdite sheep breed is among the most significant indigenous sheep breeds in Morocco because of its large population and extensive areas for breeding. A ram will have fairly well-developed spiral horns and a slender tail. Timahdite is found in the Middle Atlas Mountains of Morocco, the eastern hills, the central hills, the southeastern oasis, and the centre hills. A hybrid of the Berbère and Tadla breeds, this variety's ancestry also includes the BeniGuil, which developed the Timahdit (BOUJENANE & PETIT, 2016; BELHAJ et al., 2021).

Sardi. Sardi, which are thin-tailed sheep, are primarily used for two purposes: mainly meat production and limitedly milk production. Due to its popularity in religious and social events, the Sardi breed of sheep has developed a unique phenotype known as Chatbi. This phenotype is defined by a clean white coat and open, spiral-shaped horns (BELHAJ et al., 2021). Sardi sheep typically have a white top half and black accents around the eyes, lips, and nose. The legs are nude and the body is covered in white fleece (BOUJENANE & PETIT, 2016).

2.6.4. Breeds from Romania

Botosani Karakul. The proliferation of Karakul sheep has been a tradition in this Romania since the early 19th century, when this breed was initially imported from Russia. The nucleus, initially established and later added with flocks from Germany, Austria, and Uzbekistan, as well as local Turcana sheep (black and grey varieties), has undergone an extensive process to develop a well-adapted Karakul suited to Romania's pedoclimatic conditions, resulting in high-quality buckling. The Botosani Karakul breed was officially approved in 1988, after an extensive and complex process spanning over 50 years to establish this breed. It is a breed specialised in leather production; nonetheless, their meat production capabilities should not be overlooked due to their proven characteristics in this area (PASCAL, 2011; CRÎȘMARU et al., 2022).

Romanian Racka. The Racka breed is a transboundary breed that has diminished in popularity compared to other breeds in Romania. It is mostly farmed in limited

locations inside the Banat region, close to the Serbian border. The cause of this problem is that the Racka breed lacks economic competitiveness, fails to endure the severe winters in open fields, and has lower production levels of wool, milk, and meat compared to other selectively bred breeds (GEORGESCU et al., 2016). Racka is classified as endangered, having a population of fewer than 3,000 breeding ewes (KUSZA et al., 2017).

Transylvanian Merino. The Transylvanian Merino is a relatively newly established breed, that originally a wool breed, but has since evolved into a more versatile breed, exhibiting superior capabilities for meat and fleece production, albeit it requires more stringent feeding and housing conditions. The three breeds represent 85.4% of the total sheep population in Romania. The selection for this breed was conducted utilising its performance records through classical methodology, resulting in significant heterogeneity in production attributes (GAVOJDIAN et al., 2010; GRAS et al., 2017).

Romanian Tsigai. The Tsigai constitutes 24.3% of the total sheep population, making it the second most significant sheep breed in Romania. However, until 1989, it was mostly utilised for wool production, in addition to milk and meat. Currently, the Tsigai sheep is a multipurpose breed mostly aimed for cheese production. Tsigai breeds are raised extensively in mountainous and sub-mountainous regions characterised by broad pasturelands. The Tsigai sheep, being a rustic breed, has been the subject of research aimed at enhancing milk and meat output, predominantly by industrial crossbreeding with specialised foreign breeds (ILISIU et al., 2013; ILISIU et al., 2018).

Turcana. The Romanian indigenous Turcana breed, is a prominent representative of the Eastern European Zackel group. The substantial population, varied geographical conditions, and divergent selection practices have resulted in significant within-breed phenotypic heterogeneity. The Turcana breed encompasses five recognised varieties/ecotypes, one of which, Creata de Caransebes, is classified as endangered and included in a conservation program. The Breaza of Petrosani and Transhumant of Sibiu are the most productive and widely distributed variants (KUSZA et al., 2015).

3. MATERIALS AND METHODS

The research was authorised by the University of Debrecen's local ethics committee (registration number 19/2023/DEMÁB). All procedures adhered to the standards set out by the ARRIVE guidelines and the European Union's Animal Experimentation Directive (Directive 2010/63/EU).

3.1. Polymorphism study of heat resistance and production traits related genes across various sheep breeds

3.1.1. Sample collection and genomic DNA extraction

Blood samples were taken from 720 sheep in total. The veterinarian collected the samples in tubes containing EDTA anticoagulant. Originating from four separate countries, these animals represented seventeen distinct breeds that had adapted to certain environments (Table 2).

Table 2. Samples' origin and breed characteristics

Country origin	Breed	Tolerance to	Geographic origin	Sample size	Sampling tissue
Hungary	Suffolk	Cold	Lowland	30	Blood
	Bábolna Tetra	Cold	Lowland	36	Blood
	Ile de France	Cold	Lowland	33	Blood
	Hungarian Tsigai	Cold	Lowland	41	Blood
	Hungarian Racka	Cold	Lowland	48	Blood
	Hungarian Merino	Cold	Lowland	35	Blood
	Hungarian Awassi	Heat	Lowland	40	Blood
Bosnia & Herzegovina	Pramenka	Cold	Highland	37	Hair follicle
Morocco	Béni Guil	Heat	Lowland	30	Blood
	D'man	Heat	Lowland	30	Blood
	Timahdite	Heat	Highland	30	Blood
	Sardi	Heat	Highland	30	Blood
Romania	Botosani Karakul	Heat	Lowland	58	Hair follicle
	Romanian Racka	Cold	Highland	62	Hair follicle
	Transylvanian Merino	Heat	Lowland	60	Hair follicle

Country origin	Breed	Tolerance to	Geographic origin	Sample size	Sampling tissue
	Romanian Tsigai	Heat	Lowland	60	Hair follicle
	Turcana	Cold	Highland	60	Hair follicle

Most of the breeds are local to the countries where they originated. However, a few exotic varieties were also included to see if their adaptation was influenced by acclimatisation. Table 3 shows that the climatic conditions present during sample collection were considered together with the breed's origin and developmental history within the particular country when assessing its hot or cold tolerance trait.

Table 3. Sampling region climatological details

Sampling localization	Breeds	Altitude (m)	Temperature (°C)	
			Min	Max
Szendrő, Hungary	Suffolk Bábolna Tetra Ile de France	147	-7.6	24.9
Hortobágy, Hungary	Hungarian Tsigai Hungarian Racka	85	-7.9	25.3
Karcag, Hungary	Hungarian Merino	79	-6.5	25.8
Bakonszeg, Hungary	Hungarian Awassi	82	-7.9	25.3
Botoșani, Romania	Botosani Karakul	198	-6.0	27.0
Caraș-Severin, Romania	Romanian Racka Turcana	1251	-6.0	19.0
Baia Mare, Romania	Transylvanian Merino	256	-5.0	26.0
Arad, Romania	Romanian Tsigai	90	-2.0	28.0
Timis, Romania	Turcana	116	-4.0	28.0
Dub, Mount Vlašić, Bosnia and Herzegovina	Pramenka	654	-11.0	22.0
Eastern region of Morocco	Béni Guil	542-1706	2.0	38.0
Central plateau of Morocco	Sardi	369-793	-2.0	37.0
Oases of the South of Morocco	D'man	1026-1133	3.0	39.0

Sampling localization	Breeds	Altitude (m)	Temperature (°C)	
			Min	Max
Middle Atlas of Morocco	Timahdite	1818	-2.0	34.0

The Food and Agriculture Organization of the United Nations/International Atomic Energy Agency (FAO/IAEA) (2004) recommended method for hair follicle extraction and the ZSOLNAI and ORBÁN method (1999) for blood were employed for genomic DNA isolation (Figure 6).

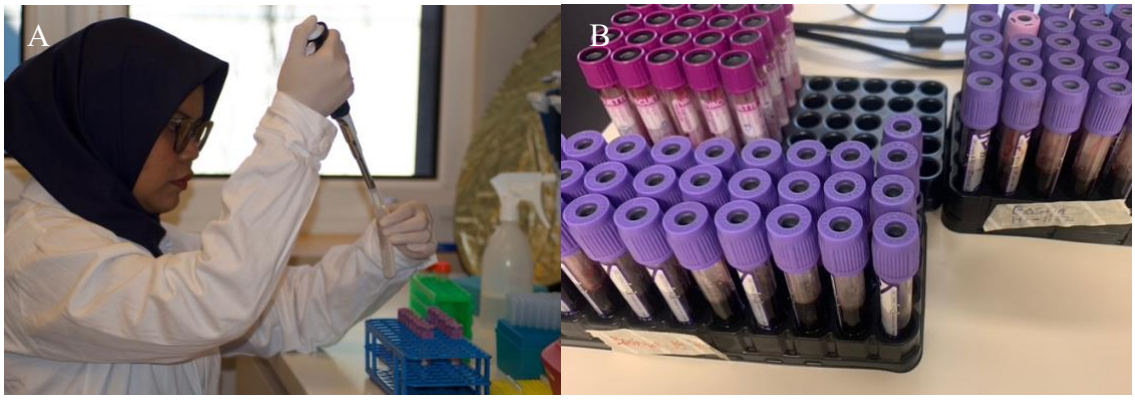

Figure 6. Laboratory work. (A) Sample preparation process at the laboratory. (B) Blood sample was stored in EDTA tube.

Before analysis, the DNA was kept at a temperature of -20 °C. The NanoDrop Spectrophotometer (Thermo Scientific, Waltham, MA, USA) was used to measure the DNA concentration. As much as 50 ng of DNA per sample was used for genotyping after diluting all of the samples to a comparable concentration.

3.1.2. Selection of SNPs

A panel of 51 SNPs representing 30 HS-related genes, spread over 18 chromosomes, was chosen based on the results of prior marker-assisted selection and genome-wide association studies (GWAS) in sheep (SINGH et al., 2017; CAVALCANTI et al., 2017; LI et al., 2019b; AL-THUWAINI et al., 2020; YOUNIS et al., 2020) (Table 4). The Ovis SNP data was obtained from the Single Nucleotide Polymorphism Database (dbSNP), which is managed by either Ensembl or the National Center for Biotechnology (NCBI) (Table S1).

Table 4. Selected SNPs used in this study

SNP	Locus	Gene name	Allele substitution	Chromosome
rs593507294	*LEP*	Leptin	C/T	4
rs161110765	*SOCS3*	Suppressor of cytokine signaling 3	A/C	11
rs161286575	*PPARG*	Peroxisome proliferator-activated receptor gamma	C/T	19
rs603870279	*ASIP*	Agouti signaling protein	C/T	13
rs598380853	*ASIP*	Agouti signaling protein	C/G	13
rs601650611	*ASIP*	Agouti signaling protein	C/G	13
rs420959261	*CSN1S1*	Casein alpha s1	C/T	6
rs587905107	*CSN1S1*	Casein alpha s1	C/T	6
rs416941267	*CSN2*	Casein beta	G/T	6
rs430298704	*CSN2*	Casein beta	C/T	6
rs420611298	*ABCG1*	ATP binding cassette subfamily G member 1	G/T	1
rs159956881	*ABCG2*	ATP binding cassette subfamily G member 2	A/G	6
rs159876394	*IGF1*	Insulin like growth factor 1	C/G	3
rs160257833	*ESR1*	Oestrogen receptor 1	A/G	8
rs591182158	*ESR1*	Oestrogen receptor 1	A/G	8
rs598908205	*GNRH1*	Gonadotropin releasing hormone 1	C/T	2
rs411181557	*DIO2*	Deiodinase iodothyronine type II	C/G	7
rs414917134	*BTNL2*	Butyrophilin like 2	C/G	20
rs405270595	*BTN1A1*	Butyrophilin	A/G	20
rs161146164	*GHR*	Growth hormone receptor	G/T	16
rs55631463	*GHR*	Growth hormone receptor	A/G	16
rs407318935	*STAT1*	Signal transducer and activator of transcription 1	A/G	2
rs161691559	*HSP90AB1*	Heat shock protein 90 alpha family class B member 1	A/G	20
rs397514115	*HSP90AA1*	Heat shock protein 90 alpha family class A member 1	G/C	18

SNP	Locus	Gene name	Allele substitution	Chromosome
rs397514116	*HSP90AA1*	Heat shock protein 90 alpha family class A member 1	C/G	18
rs397514117	*HSP90AA1*	Heat shock protein 90 alpha family class A member 1	A/C	18
rs397514269	*HSP90AA1*	Heat shock protein 90 alpha family class A member 1	A/G	18
rs397514270	*HSP90AA1*	Heat shock protein 90 alpha family class A member 1	G/T	18
rs397514271	*HSP90AA1*	Heat shock protein 90 alpha family class A member 1	A/G	18
rs397514268	*HSP90AA1*	Heat shock protein 90 alpha family class A member 1	-/G	18
rs397514272	*HSP90AA1*	Heat shock protein 90 alpha family class A member 1	G/T	18
rs397514273	*HSP90AA1*	Heat shock protein 90 alpha family class A member 1	A/G	18
rs588145625	*HSPA8*	Heat shock protein family A member 8	A/G	15
rs161504783	*HSPA12A*	Heat shock protein family A member 12A	C/T	22
rs160077209	*HSPA4*	Heat shock protein family A member 4	A/G	5
rs589164764	*IL1R1*	Interleukin 1 receptor type 1	C/T	3
rs160387232	*IL1R1*	Interleukin 1 receptor type 1	C/T	3
rs590620426	*IL2*	Interleukin 2	C/G	17
rs596312311	*IL2*	Interleukin 2	C/T	17
rs416425182	*TR*	Thyroglobulin	A/C	9
rs595200178	*TR*	Thyroglobulin	A/G	9
rs418400798	*TR*	Thyroglobulin	C/T	9
rs410259751	*IL33*	Interleukin 33	G/T	2

SNP	Locus	Gene name	Allele substitution	Chromosome
rs162295351	*HSP90AB1*	Heat shock protein 90 alpha family class B member 1	A/C	20
rs161691552	*HSP90AB1*	Heat shock protein 90 alpha family class B member 1	A/G	20
rs597293577	*STAT_PIAS3*	Protein inhibitor of activated STAT 3	C/T	1
rs593155540	*STAT_PIAS3*	Protein inhibitor of activated STAT 3	A/G	1
rs602521720	*HCRT*	Hypocretin neuropeptide precursor	C/G	11
rs425706327	*USP19*	Ubiquitin specific peptidase 19	A/G	19
rs161274296	*USP19*	Ubiquitin specific peptidase 19	G/T	19
rs588498137	*STAT3*	Signal transducer and activator of transcription 3	A/G	11

3.1.3. Genotyping and ensuring product quality

With the help of Kompetitive Allele Specific PCR (KASP™, LGC Genomics, Teddington, Middlesex, UK), the 51 SNPs that were chosen for bi-allelic discrimination were tested. For viewing the results, we utilized SNP Viewer software, version 1.99 (Hoddesdon, UK) and from there, the genotype data was exported for the purpose of statistical analysis. To focus the results, data only included SNPs that were present in half or more of the breeds. Animals and SNPs with a call rate below 50% were removed as part of the data quality control process for genotyped data. The number of animals per breed and the number of SNPs per animal were both affected by this.

3.1.4. Data analysis

The raw allele calls acquired from LGC Genomics were examined utilizing LGC Genomics' KlusterCaller software. Utilising LGC Genomics' KlusterCaller software, the raw allele calls obtained from LGC Genomics were evaluated. Utilising POPGENE software version 1.31 (YEH et al., 1999), the gene diversity, allele and genotype frequencies, and compliance with or deviation from the Hardy-Weinberg equilibrium were evaluated.

Principal Component Analysis (PCA) was conducted utilizing the FactoMineR (LÊ et al., 2008) and ggplot2 (WICKHAM, 2016) packages from the R Program (R CORE TEAM, 2020) to illustrate the genetic diversity among sheep breeds categorized by their

climatic adaptations which was determined based on breed's origin, breed history/formation and sampling location: cold-tolerant breeds (Bábolna Tetra, Hungarian Merino, Hungarian Racka, Hungarian Tsigai, Ile de France, Pramenka, Romanian Racka, Suffolk, and Turcana), heat-tolerant breeds from Morocco (Béni Guil, D'Man, Timahdite, and Sardi), and heat-tolerant breeds raised in Europe (Hungarian Awassi, Botosani Karakul, Transylvanian Merino, and Romanian Tsigai).

3.2. Relative expression levels of heat stress-related genes in sheep in different seasons

3.2.1. Sample collection and sampling location

A total blood sample (Figure 7) was initially obtained from 24 animals (12 ewes and 12 rams) representing three distinct breeds: Hungarian Merino, Hungarian Indigenous Tsigai and White Dorper. The three breeds represent the main sheep breed kept in Hungary, the indigenous sheep, and the imported breed, respectively.

Figure 7. Sampling process. (A) Blood collection through the jugular vein. (B) Blood sample was stored in Tempus™ Blood RNA Tubes

There were no morphological or anatomical anomalies among the animals that participated in the study, and they were all of similar age (2 to 3 years), weight (ewes: 45 to 55 kg; rams: 65 to 75 kg), and perfect health. The University of Debrecen's Kismacs Experimental Station of Animal Husbandry, which is located at 47.58° N and 21.58° E, and 127 meters above sea level, was used to keep the animals. Average yearly precipitation is between 550 and 600 millimetres, while average yearly high and low temperatures are between -7.5 and 28.0 °C. Throughout 2019 and 2020, the sample was taken in four distinct seasons: spring (April), summer (August), autumn (November), and winter (January). Throughout the duration of the trial, all animals were treated in the same

manner. Within the sheep shed system, which consisted of an enclosure, the breeds were cared for and fed together all year round, with sex divisions made. Their confinement in harems was a prerequisite for mating in the autumn. Every animal has unlimited access to fresh water and a diet of hay and fodder, with each sheep receiving 0.4 kg/day. Compost had 50% maize and 50% oats. An additional kg of alfalfa hay is given to ewes when they are lambing. Every day for four weeks leading up to insemination and throughout the procedure, each sheep was given 1 kg of alfalfa silage. The animals had access to selenium lick blocks for one whole year.

As a result of the long research duration, several animals were excluded from the target population during implementation, so decreasing the total sample size to 15 animals: Hungarian Merino (2 ewes and 1 ram), Hungarian indigenous Tsigai (3 ewes and 3 rams), and White Dorper (2 ewes and 4 rams). About 5 ml of blood was drawn from the jugular vein of the same animals throughout each peak season using Tempus™ Blood RNA Tubes (Applied Biosystems, USA) then kept at a temperature of -70°C for further examination.

3.2.2. Climatological data

Hourly weather reports were kept on the day of the sample (Table 5). The THI was determined daily by applying the formula proposed by MADER et al. (2006). Animals' HS severity is measured using the THI, which goes from 0 (no stress) to >84 (extreme stress), with the following categories: THI≤67 no stress, THI 68-74 mild, THI 75-78 moderate, THI 79-83 severe and THI≥84 extreme (LEWIS BAIDA et al., 2021).

$$THI = (0.8 \times T_{db}) + \left[\left(\frac{RH}{100}\right) \times (T_{db} - 14.4)\right] + 46.4$$

T_{db}- Dry bulb temperature (°C), RH- Relative humidity (%)

Table 5. Climatic conditions of sampling days

Season	Date	Time	Temperature (°C)	Relative humidity (%)
Spring	29/04/2020	12.00-13.30	19.31	62.66
Summer	13/08/2019	12.00-13.00	32.80	34.50
Autumn	19/11/2020	12.00-13.30	7.88	99.84
Winter	22/01/2020	12.00-13.30	-3.33	99.70

3.2.3. Quantification of gene expression levels using qRT-PCR

Following the manufacturer's instructions, 3 ml of total blood was used to isolate total RNA using the Tempus Spin RNA Isolation Kit (Applied Biosystems, USA). DNase (Quiagen, catalogue number: 79256) was then added.

The NanoDrop ND-1000 Spectrophotometer (Thermo Fisher Scientific Waltham, MA, USA) was used to evaluate RNA's amount and quality. Using the qPCRBIO cDNA Synthesis Kit (PCR Biosystems, London, United Kingdom), 3000 nanogrammes of total RNA were reverse transcribed into complementary DNA with the help of specific primers listed in Table 6. For every real-time PCR run, a volume of cDNA equal to 5 ng of starting total RNA was utilised as the template. To construct the forward and reverse primers, Primer Express v3.0.1 software (Applied Biosystems Foster City, CA, USA) was used. Followed with Primer Blast from NCBI (YE et al., 2012) to check their target identification. The Roche Light Cycler 96 Real-Time PCR System was employed for qPCR, consisting of a 3-minute denaturation phase, followed by 50 cycles at 95 °C for 15 seconds, 62 °C for 20 seconds, and 72 °C for 15 seconds. High-resolution melting analysis was conducted for each run (*GAPDH*= 83.0°C, *IL10*= 87.0°C, *TLR2*= 82.5°C, *TLR4*= 80.5°C, *TLR8*= 80.0°C, and *HSP70*= 84.0°C).

Table 6. Detailed list of primers for qRT-PCR analysis of target genes

Target gene	GenBank accession	Primer	Amplified product size (bp)
HSP70	NC_056073.1	F: GGAGTCGTACGCCTTCAACA R: CACCTTCTTCTTGTCCGCCT	85
IL10	NC_056065	F: TGATGCCACAGGCTGAGAAC R: CAGAAAACGATGACAGCGCC	110
TLR2	NM_001048231.1	F: ACTCCATCCCCTCTGGTCTC R: CAGGTCTCTGTTGCCGACAT	86
TLR4	NM_001135930.1	F: GGTGGAGCTCTATCGCCTTC R: GGTGCGTTACCCCTGCTATT	77
TLR8	NM_001135929.1	F: CAGTGAGTTGCCGTTGTTGG R: GGTGCGTTACCCCTGCTATT	77
GAPDH	NC_040254.1	F: CTGGCCAAGGTCATCCAT R: ACAGTCTTCTGGGTGGCAGT	86

The reactions were initiated according to the manufacturer's directions using PowerUpTM SYBR! Green Master Mix (Applied Biosystems Foster City, CA, USA). Each real-time PCR experiment employed a template quantity of cDNA that was 5 ng of starting total RNA.

A housekeeping gene (*GAPDH*) was amplified in conjunction with the target genes (*IL10*, *TLR2*, *TLR4*, *TLR8*, and *HSP70*) in order to do relative gene expression assays. For every sample, quantitative PCR was performed three times.

3.2.4. Statistical analysis

The relative quantification of the target gene was assessed using the Pfaffl technique (PFAFFL, 2001). The reference gene GAPDH was compared to the GOI of HSP70, IL10, TLR2, TLR4, and TLR8, with the spring season's value serving as the calibrator. Primers' efficacy was determined using LinReg PCR version 2017.0 (RAMAKERS et al., 2003). SPSS Version 25 (IBM Corp., Armonk, NY, USA) was used to conduct the analyses with a mixed-model ANOVA with repeated-measures GLM. The assumption of sphericity in the repeated measurement was decided using Mauchly's test, and the median for the equality of error variance within season measurement was utilised in Levene's test. Additional testing involved applying the Tukey test for post hoc multiple comparisons. GraphPad Prism for macOS, version 8.0.0 (GraphPad Software, San Diego, CA, USA), was used to create the data visualisations. The mean ± SD is used to display the results. Statistical significance was established when a difference had a p-value less than 0.05.

3.3. Gene expression study in white-coated and black-coated sheep using RNA-Sequence method

3.3.1. Sample collection

This study used a total of 10 Hortobágyi Racka ewes; 5 individuals with white-coated and 5 individuals with black-coated (Figure 8). All the sheep involved in the study were of similar age, ranging from 1.5 to 2 years old, and had a body weight between 50 to 60 kg. All animals were raised under identical environmental circumstances and feeding at Hortobágyi Nonprofit Kft. Sampling was done in early summertime (30 May 2023) with the average temperature during the sampling period (09.00-13.00) ranging between 17.1 – 22.5°C.

Figure 8. Black Hortobágyi Racka and white Hortobágyi Racka sheep before slaughtering and sampling process

Skin samples were collected from the back of the animal's neck immediately following the slaughtering process, within a maximum of one hour post-mortem and were preserved in RNAlater™ Solution (Thermo Fisher Scientific, Waltham, MA, USA) (Figure 9) and stored temporarily in ice box with 4 °C during the transportation to the laboratory and subsequently stored in a freezer at −70 °C until RNA isolation.

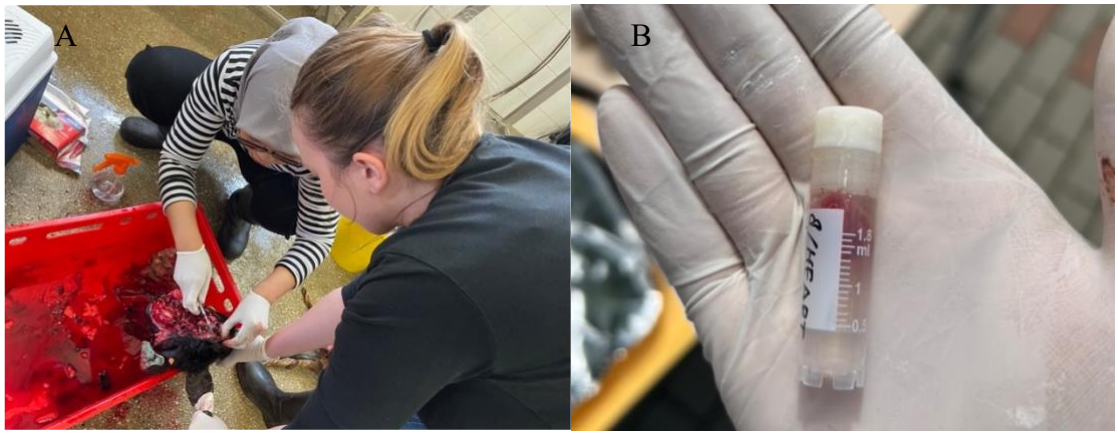

Figure 9. Postmortem sampling at the slaughterhouse. (A) Cutting skin samples from the back of the head. (B) Sample was preserved in the RNAlater™ Solution

3.3.2. RNA extraction, library preparation and sequencing

Total RNA was extracted from skin sample with Direct-zolTM-96 MagBead RNA Kit (Zymo Research, Orange, CA, USA) according to the manufacturer's instructions. The QuantSeq 3' mRNA-Seq Library Prep Kit FWD for Illumina (Lexogen, Vienna,

Austria) was used to create the RNA libraries. To synthesise first-strand cDNA with an oligodT primer and then remove RNA, 100 ng of total RNA was utilised as an input. After that, magnetic beads were used to purify the products, and random priming was used to begin the second strand synthesis. The libraries were then barcoded and amplified using PCR. Each library was tested on an Agilent 4200 TapeStation (Agilent Technologies, Palo Alto, CA, USA) to see whether adapter dimers were produced during PCR. The QuantSeq libraries were sequenced using the Illumina NovaSeq 6000 platform (Illumina, San Diego, CA, USA) with 2 ×151 run configuration.

3.3.3. Bioinformatics analysis

Using the bcl2fastq v2.20.0.422 software (Illumina Inc.), the sequencing instrument's Binary Base Call (BCL) files were base called, demultiplexed, and converted into FASTQ files. The reads were then quality-trimmed to Q30 and trimmed to 1x75 bp (minimum length: 40 bp) using the Phred algorithm with BBDuk from the BBTools suite v38.86 (BUSHNELL et al., 2017).

The processed reads were aligned against the domestic sheep (*Ovis aries*, Rambouillet breed, OAR_USU_Benz2616 strain) reference genome downloaded from NCBI (ARS-UI_Ramb v30 assembly; Jul 20, 2023; RefSeq: GCF_016772045.2) with STAR v2.7.6a (DOBIN et al., 2013). Using the HTSeq Python package v0.11.1 (ANDERS et al., 2015), the amount of reads aligned inside each gene was counted. A normalisation procedure called the trimmed mean of M values (TMM) was applied to the gene count data using the edgeR R/Bioconductor package (v3.28, R v3.6.0, Bioconductor v3.9). Data were further log transformed using the Voom approach for statistical evaluation in the limma package (RITCHIE et al., 2015), which also used to compute the fold change (FC) values between the groups that were compared, as well as the p-values for the moderated t-test. Values expressed as transcripts per million (TPM) were used to normalise counts in exploratory data analysis and visualisation.

3.3.4. Function enrichment analysis

Using the DAVID Database (https://david.ncifcrf.gov/tools.jsp; DENNIS et al., 2003), the differentially expressed genes (DEGs) were analysed for gene ontology (GO) categories: biological process (BP), molecular function (MF), and cellular component (CC). GO keywords or KEGG pathways were considered significant if their corrected P-values (based on false discovery rate, FDR) were below 0.05. The bubble plot for the

KEGG pathway enrichment was constructed using the ggplot2 package (WICKHAM, 2016) in R program. Protein-protein interaction (PPI) networks for the DEGs were built in STRING Database (https://string-db.org).

3.3.5. Validation of differentially expressed genes

As a result of this study, a total of 10 genes (Table 7) were chosen for further investigation and comparison of gene expression in skin tissue of black coated and white coated Hortobágyi Racka sheep using qRTP-CR. These genes were selected based on their significant differential expression in the RNA-Seq analysis and has been reported to be significantly associated with immunity, metabolism pathways, and skin pigmentation. Primer Express v3.0.1 software (Applied Biosystems, Foster City, CA, USA) was used to design the primers that were used to convert the total RNA sample, which included 300 ng, into cDNA. Primer Blast from NCBI (YE et al., 2012) was used to verify the primers' target specificity.

The qPCRBIO cDNA Synthesis Kit (PCR Biosystems, London, UK) was used for this process. For each real-time PCR experiment, the template consisted of a quantity of cDNA equivalent to 5 ng of the initial total RNA. The Roche Light Cycler96, a Real-Time PCR System, was utilized for qPCR. The process involved a 3-minute denaturation step, followed by 50 cycles of 95 °C for 15 seconds, 62 °C for 20 seconds, and 72 °C for 15 seconds. Each run underwent high resolution melting analysis.

Applied Biosystems' PowerUpTM SYBR! Green Master Mix (Foster City, CA, USA) was used to prepare the reactions in accordance with manufacturer instructions. A quantity of cDNA equivalent to 5 ng of the starting total RNA served as the template for every real-time PCR assay.

In order to conduct relative gene expression investigations, the amplification of a housekeeping gene, *GAPDH*, was performed in conjunction with the 10 target genes*: IRF4, PMEL, GPNMB, TYRP1, PLXNC1, VNN1, TYR, PLP1, SLC24A5, and TRPM1*. Each sample was subjected to quantitative PCR in triplicate. Changes in gene expression (CT value) were calculated using the $2^{-\Delta\Delta Ct}$ (DDCT) method, the fold changes were analysed with Independent Sample T-Test.

Table 7. Primers details

Target gene	GenBank accession	Primer	Amplified product (bp)
IRF4	XM_060403818.1	F: TATCAGTGTCCCGTGACCTTC	78
		R: TGTGACCTGGCAACCATTTTC	
PMEL	XM_004006599.6	F: GCCCTTCTGGCCCTTTATCT	73
		R: ACTTGCCAGTATTGGTCCCAG	
GPNMB	XM_004007790.6	F: ACCACCCCTTCTTTGGTAACTG	61
		R: CATCAGGAATCTCCCTCAGCTC	
TYRP1	XM_042242266.2	F: AAGTTCAATGGCCAGGTCGG	72
		R: TATCCAGAGCCTGGACAAAGC	
PLXNC1	XM_015094600.3	F: TGGAGCTAAAAAGTGCCCTCAA	68
		R: CCACCGTCACTGTCGTCTTAT	
VNN1	XM_004011337.6	F: GAGAAGGATAGTGCACCAAATGC	69
		R: TGAGCATCACAAATACCTTAGCG	
TYR	NM_001130027.1	F: CAGGTTCCATGGATAAAGCTGC	66
		R: GCGGACTAGCAAATCCTTCCA	
PLP1	XM_004022464.6	F: CCAGCAAGACCTCTGCAAGTA	70
		R: TTCCATGGGAGAACACCATACA	
SLC24A5	NM_001252181.1	F: CTCCCAAGGGCTACAGGAAATAG	76
		R: TCGTGAAAAACCCCTCAGGAAA	
TRPM1	XM_060401392.1	F: ACTGCCCTGCTGAAAGGAAC	77
		R: ATGTCCACACGGTTCCAAGC	
GAPDH	NM_001190390.1	F: CATCCCTGCTTCTACTGGCG	72
		R: CCAGTGAGCTTCCCGTTGAG	

4. RESULTS

4.1. Polymorphism of heat stress-related genes in heat tolerance and cold tolerance sheep breeds

4.1.1. Allele and genotype frequency

In light of the potential increase in global temperatures due to climate change, the genetic basis of thermotolerance adaption in sheep is of major relevance for current doctoral research. The purpose of this phase of the doctoral research was to examine the genetic basis of thermotolerance adaptability of different breeds from different geographical areas.

Out of a total of 720 animals, 601 (83.47%) were successfully genotyped. Damage to samples during transportation to the subcontracting laboratory may account for the comparatively high percentage of failed genotypes (37.26%). In addition, there is less data available for analysis due to quality control. Of the 51 SNPs initially examined, 32 (62.74%) were successfully genotyped, 17 of these were polymorphic *HSPA12A, HSP90AA1, IL33, DIO2, BTNL2, CSN2, ABCG1, CSN1S1, GHR, HSPA8, STAT3*, and *HCRT*. Table S2 in the appendix shows that the frequencies of certain alleles and genotypes varied between populations.

All breeds were successfully genotyped for four SNPs: rs161504783-*HSP12A*, rs588145625-*HSPA8*, rs588498137-*STAT3* and rs602521720-*HCRT*. However, rs588498137-*STAT3* and rs602521720-*HCRT* did not show any trends in genotypic or allelic frequencies, thus the attention was focused on *HSPA12A* and *HSPA8*, two SNPs that showed patterns of allelic and genotypic frequencies in relation to climatic characteristic.

All breeds except Sardi, Hungarian Merino, Botosani Karakul and Transylvanian Merino exhibited dominant heterozygote *TC* for rs161504783-*HSPA12A*. On the whole, the frequencies of the *T* and *C* alleles were nearly similar in most breeds. However, two notable exceptions were the Sardi and Botosani Karakul, where the *T* allele frequency was 0.735 and the *C* allele frequency was 0.265, respectively. Cellular senescence and heat stress responses are two aspects that *HSPA12A* influences (VANSELOW et al., 2016). Ruminants are more likely to exhibit this trait during the summer, which may help them survive in harsh environments. Indicine cattle (*Bos taurus indicus*) have a strong correlation with heat tolerance, and this gene plays a significant role in water holding capacity in beef cattle breeds. It is possible that this gene helps animals survive heat stress

by regulating muscle pH and increasing adrenaline levels (JOY et al., 2020a; SARAVANAN et al., 2021).

Aside from the Transylvanian Merino, no heat-tolerant breeds or those with cold tolerance have the heterozygote *GA* for SNP rs588145625-*HSPA8*. These breeds include the Hungarian Racka, Bábolna Tetra, Hungarian Tsigai, Romanian Racka, Pramenka, and Turcana. No heat-tolerant breed was found without the homozygote *GG*, and even several cold-tolerant ones (the Suffolk, the Ile de France, and the Hungarian Merino) have it. With frequencies ranging from 0 to 0.308, the *A* allele has only been found in cold-tolerant breeds and Transylvanian Merino, while the *G* allele was dominant across all breeds, with frequencies ranging from 0.760 to 1.

According to SINGH et al. (2014) and HOTER et al. (2018), *HSPA8* is involved in several regular physiological processes, including protein folding and unfolding, preventing polypeptide aggregation, disassembling large protein complexes, and translocating proteins across different parts of the cell. Many livestock species have used the *HSPA8* gene as a potential heat resistance gene because it has been found to be positively associated with heat tolerance (VERMA et al., 2015; AL-THUWAINI et al., 2020; ONASANYA et al., 2021).

For SNP rs588498137-*STAT3*, the *G* allele and *GG* genotype were dominant across all breeds, with a *G* allelic frequency ranging from 0.750 to 1. Similarly, for SNP rs602521720-*HCRT*, the *C* allele and *CC* genotype were dominant across all breeds, with a *C* allele frequency ranging from 0.546 to 1.

Allele frequencies, observed and anticipated genotypes, and the P-values of the polymorphic genes are summarised in Appendix Table S2, which was used to evaluate the HWE with a Chi-square (x^2) test. The breed with the highest number of SNPs that deviated from HWE ($P \leq 0.05$) was determined to be the Romanian Botosani Karakul; rs416259751-*IL33*, rs411181557-*DIO2*, rs414917134-*BTNL2* and rs420611298-*ABCG1*. This might be due, in part, to the fact that, since its arrival from Russia in the early 1800s, this breed has been heavily hybridized with other Romanian breeds as well as those from Germany and Austria (PASCAL, 2011; KEVORKIAN et al., 2011).

4.1.2. Genetic diversity and interrelationship between SNPs

PCA was performed using SNPs data to show how the breeds (Figure 10a) and climatic characteristics (Figure 10b) differed. Both figures show that of the overall variation in the 17 breeds, 11.21% is accounted for by the PC1, and 9.98% by the PC2.

Even after we separated the heat-tolerant breeds raised in the European Union and Morocco, neither PC1 nor PC2 could distinguish between the breeds or the climate regions. The majority of breeds were found in the range of –2.50<PC1<1.12, with cold-tolerant breeds having a PC1 score ranging from –5.593 to –5.013 and a PC2 score from –4.149 to 4.020. On the other hand, heat-tolerant breeds had a PC1 score ranging from –0.452 to 0.460 and a PC2 score from 0.157 to 1.183. Breeds raised in Europe that are heat-tolerant had PC1 and PC2 scores ranging from –0.881 to –0.686 and –3.987 to 2.931, respectively.

This could be because hot-tolerant breeds kept in the EU have adapted to the subtropical climate, and because genetic admixture has happened due to reproductive technologies. As a result, there is less clear genetic divergence between breeds, as also reported in previous studies discovered high levels of admixture between various sheep breeds raised in Romania and Hungary, with the goal of increasing productivity (KUSZA et al., 2009; KUSZA et al., 2010; KUSZA et al., 2015; GAVOJDIAN et al., 2015a; GAVOJDIAN et al., 2016).

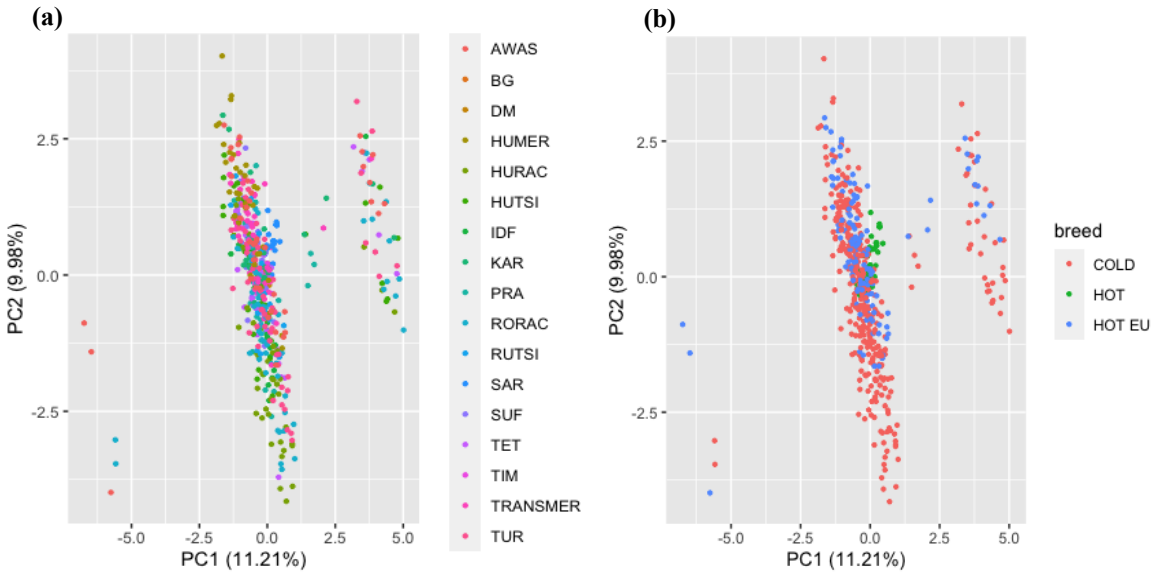

Figure 10. Score biplot of Principal Component Analysis of the 17 SNPs and 601 animals

(a) Individuals of different breeds are differently colored; (AWA: Hungarian Awassi, BG: Béni Guil, BTET: Bábolna Tetra, DM: D'Man, HUME: Hungarian Merino, HUTS: Hungarian Tsigai, IDF: I de France, KAR: Botosani Karakul, PRA: Pramenka, ROME: Transylvanian Merino, RORA: Romanian Racka, ROTSI: Romanian Tsigai, SAR: Sardi, SUF: Suffolk, TIM: Timahdite and TUR: Turcana). (b) Breeds grouped by climatic characteristics; COLD: cold-tolerant breeds (Suffolk, Bábolna Tetra, Ile de France, Hungarian Tsigai, Hungarian Racka, Hungarian Merino, Pramenka, Romanian Racka, and Turcana), HOT: heat tolerant breeds originated from Morocco (Béni Guil, D'Man, Timahdite and Sardi), HOT EU: Heat tolerant breeds reared in Europe (Hungarian Awassi, Botosani Karakul, Transylvanian Merino, and Romanian Tsigai).

The highest contributions to the principal component were by rs397514117-*HSP90AA1* (c) and rs397514272-*HSP90AA1* (e), whereas rs410259751-*IL33* (g) also had a positive contribution (Figure 11). The loading values and score values of PCA are available in Appendix Table S3 and Appendix Table S4. Previous studies have shown that *HSP90AA1* is linked to sheep's susceptibility to thermal stress (MARCOS-CARCAVILLA et al., 2009; SALCES-ORTIZ, et al., 2015). On the other hand, *IL33* has been reported to affect sheep's immunity and resistance to gastrointestinal intestinal nematode infection (ESTRADA-REYES et al., 2019; TOSCANO et al., 2019). These findings are related because heat stress increases animal vulnerability to illnesses and promotes immune suppression (CAROPRESE et al., 2021).

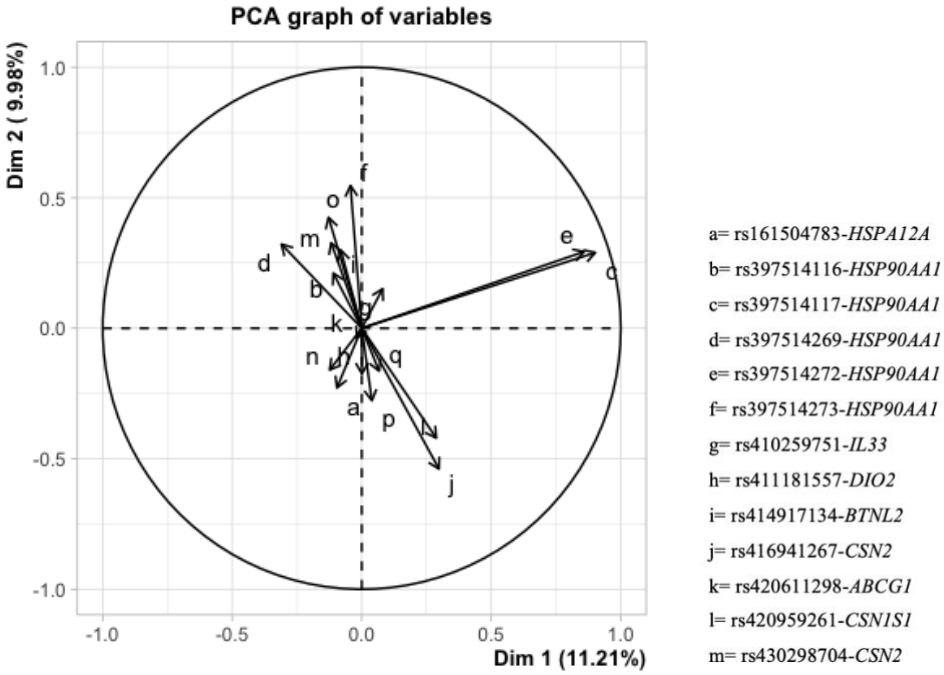

Figure 11. Loading biplot of principal component analysis of the 17 SNPs for 17 sheep breeds

4.2. Relative expression levels of heat stress-related genes in sheep in different seasons

4.2.1. Climatological conditions

Indigenous Hungarian Tsigai, commercial Hungarian Merino (abreed that has persevered over several generations of cold weather), and commercial White Dorper (a tropical originated breed with great tolerance to heat) were the three most frequent sheep

breeds in Hungary that were assessed in this study. The white coat is the most prominent feature of all the breeds studied here. The White Dorper has short wool, while the Hungarian Merino and indigenous Tsigai have long. Although all three breeds have been acclimated to the Hungarian environment due to their extensive keeping in the country, the objective was to determine whether there is a difference in their adaptability to the local climate.

Table 5 shows the weather conditions on the sampling day for each season, and Table 8 displays the calculated THI. With the exception of the summer season, when the THI reached 78.99—nearly a severe heat stress condition—all other sampling days fell inside the thermoneutral zone. Although it varies substantially by breed, age, and physiological state, the upper critical temperature of sheep usually falls between 25 and 31 °C. As per RATCHAMAK et al. (2021), the simplest method for assessing the impact of climate change on livestock production is the THI. This is calculated by summing the average temperature (°C) and relative humidity (%) of a given area. Nevertheless, it is evident that THI might not provide a complete picture of the climatic conditions under which animals live due to the absence of elements including wind speed, sun radiation, shadow and water availability.

Table 8. The THI in each sampling season

Season	THI
Spring	64.94
Summer	78.99
Autumn	46.19
Winter	26.06

4.2.2. Relative gene expression

The Pfaffl method (PFAFFL, 2001) was used to calculate the relative expression, which is displayed in Table 9. The spring season was chosen as the calibrator because it was considered to be thermo-neutral. The study found that the expression of all genes of interest (GOI) was lowest in autumn compared to spring.

Table 9. Seasonal relative gene expression calculation of *IL10, TLR2, TLR4, TLR8,* and *HSP70* of Hungarian indigenous Tsigai, Hungarian Merino, and White Dorper

Gene	Season	Relative gene expression (Mean ± SD)		
		Hungarian Tsigai	Hungarian Merino	White Dorper
HSP70	Summer	0.777±0.313	7.494±11.932	0.652±0.219
	Autumn	0.527±0.192	0.378±0.193	0.381±0.083
	Winter	0.810±0.889	2.487±2.092	0.524±0.498
	Spring	1.039±0.326	1.024±0.265	1.078±0.441
IL10	Summer	6.299±6.412	1.976±1.642	0.107±0.084
	Autumn	0.578±0.175	0.554±0.156	0.524±0.072
	Winter	0.838±0.516	2.553±2.128	1.935±2.336
	Spring	1.124±0.576	1.026±0.294	1.056±0.380
TLR2	Summer	12.053±11.018	3.317±2.720	14.263±13.417
	Autumn	0.369±0.219	0.304±0.054	0.419±0.116
	Winter	2.062±2.126	1.838±2.630	1.644±1.695
	Spring	1.028±0.261	1.019±0.231	1.107±0.420
TLR4	Summer	0.379±0.100	15.204±19.950	14.263±13.417
	Autumn	0.428±0.090	0.324±0.291	0.419±0.116
	Winter	2.283±1.817	3.414±4.913	1.644±1.695
	Spring	1.021±0.242	1.028±0.306	1.107±0.420
TLR8	Summer	5.747±5.481	7.497±6.507	6.910±8.130
	Autumn	0.257±0.085	0.842±0.353	0.228±0.080
	Winter	1.593±1.818	6.617±9.523	0.898±1.296
	Spring	1.061±0.406	1.271±1.018	1.100±0.597

SD: Standard deviation. Expression is relative to spring season as a calibrator.

The relative expression was visualized in Figure 12. For *HSP70*, relative gene expression with different peaks was observed; spring for Hungarian Tsigai (1.039±0.326) and White Dorper (1.078±0.441), summer (7.494±11.932) for Hungarian Merino. According to ROUT et al. (2017) and JOY et al. (2020a) increased HSP concentrations are consistently linked to thermal acclimation and adaptation in small ruminants. Although HSPs have been at the center of cellular responses in sheep, the process by which they tolerate heat stress is complicated and yet not completely understood. Low amounts of HSP

make cells more susceptible to heat stress, whereas high quantities of HSP improve cell response to heat stress.

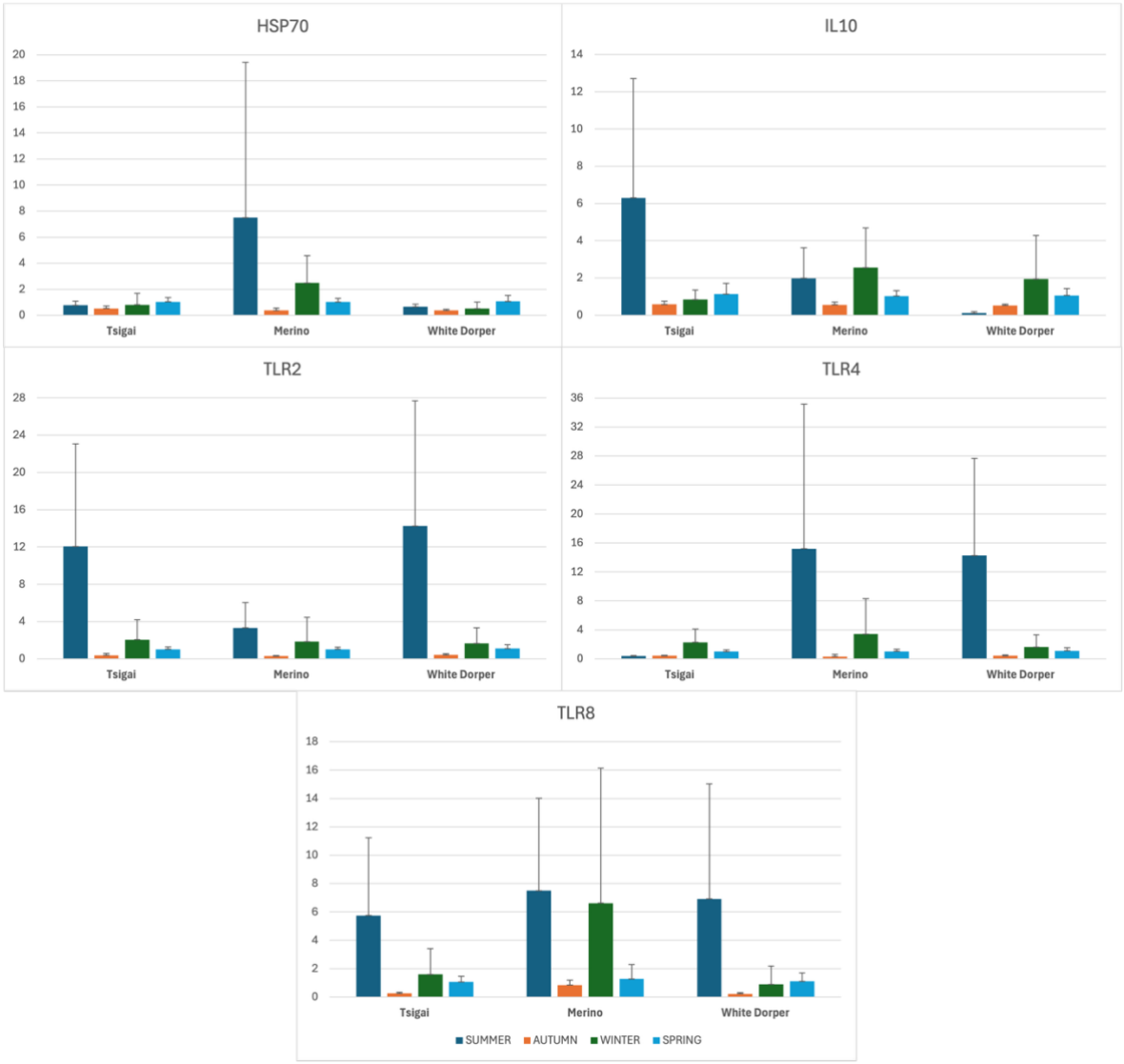

Figure 12. Bar graph of relative gene expression of *HSP70*, *IL10*, *TLR2*, *TLR4*, and *TLR8* in each season of the year with the spring season as the calibrator.

Seasonal differences in gene expression are shown on the X-axis. The Y-axis displays the gene expression levels for all breeds included in the study. The Hungarian Merino is blue, the White Dorper is yellow, and the Hungarian Tsigai is red.

A summertime increase in HSP70 relative gene expression may indicate that heat stress is a key regulator of this protein's expression. Even though the Hungarian Merino breed can withstand cold temperatures, it is very vulnerable to heat exhaustion. Research conducted by HOOPER et al. (2018) and ALEENA et al. (2018) suggests that when the

HSP70 gene expression level is higher, there is a greater requirement to protect cell protein structures against denaturation and aggregation, two types of heat stress damage.

As an intercellular function, the external *HSP70* is involved in molecular protection against heat stress, in addition to its vital role in the immune system. One pathway involves the PAMP working in collaboration with host pattern recognition molecules such as *TLRs* to initiate an immunological response, leading to an increase in the quantity of macrophages and neutrophils (DYBDAHL, 2005; HASSAN et al., 2019). The second technique works by controlling cellular pathways that indicate inflammation; this can result in the production of *IL10*, the most significant cytokine for the immune system and inflammation suppression (BORGES et al., 2012).

The *HSP70* gene is upregulated in response to heat stress, and prior research on goats demonstrated that it is critical for the adaptability and tolerance of goats to environmental stress (BANERJEE et al., 2013; ARCHANA et al., 2018). Goats differ in their heat tolerance and their capacity to adapt to diverse climates, which may explain why the *HSP70* gene expression pattern is breed-and species-specific (BANERJEE et al., 2013). Additionally, they verified that heat-adapted goats (Sihori and Barbari) have much lower summertime *HSP70* gene expression than Indian cold-adapted goats (Gaddi and Chegu). Other animals, such as buffalo (YADAV et al., 2021) and cattle (BHARATI et al., 2016), have also shown an increase in *HSP70* expression in response to heat stress. This study found that there was only a slight seasonal variation in the relative gene expression of *HSP70* between the Hungarian indigenous Tsigai and the White Dorper.

According to GÁSPÁRDY et al. (2006) and KUSZA et al. (2010, 2011, 2015), the Hungarian indigenous Tsigai sheep has long history in Hungary's agricultural tradition and economy, as well as its resilience and adaptability to extreme climates, the breed has come to prominence in Hungary. Conversely, White Dorpers are better at thermoregulation than other breeds because of morphological features that help them deal with heat stress: short hair, thin skin, and a low density of hair follicles per unit area (GOOTWINE, 2011). This goes against what is typical of other breeds that have to control their physiological reactions, such increasing their breathing and heart rates to expel internal heat or lowering their food consumption and weight to decrease endogenous heat production (MARAI et al., 2006). ALMEIDA et al. (2013) and JOY et al. (2020b) are only two of the many studies that have shown how remarkable their thermoregulation and heat stress resistance are.

There is a strong correlation between heat stress and poor health because this change causes destruction of the immune system because it shifts the adaptive immunological function from the usual cell-mediated to humoral immunity. SHI et al. (2020) and SOPHIA et al. (2016b) found that sheep's immunity is lowered and they become more disease-prone when subjected to heat stress. There is a general assumption that glucocorticoids produced by heat stress disrupt immune homeostasis via changing cytokine production. Still, the exact mechanism has not been uncovered (BAGATH et al., 2019).

The *IL10* gene showed an increase in expression during the summer for Hungarian Tsigai (6.299±6.412), but for Hungarian Merino and White Dorper, the highest expression was identified in the winter season, accounted for 2.553±2.128 and 1.935±2.336, respectively.

Studies on many cattle breeds have demonstrated that heat stress elevates *IL10* expression, namely in Karan Fries, Sahiwal cows, and Jersey (SHEIKH et al., 2016; GREWAL et al., 2019; KIM et al., 2020). Similarly, in sheep, hyperthermia increases plasma *IL10* production (CAROPRESE et al., 2014), which is thought to be a mechanism for the immune system to adapt better to heat stress. This finding is in line with that of RASHAMOL et al. (2019), who found no statistically significant difference in *IL10* expression between heat-stressed and non-stressed Malabari goats. The Hungarian Merino in this study, showed remarkable resilience to heat stress, particularly in terms of their capacity to sustain the innate immune response. The Merino sheep had plenty of time to adjust to the semi-arid environment and pastures of Hungary after their initial introduction in the 17th century, according to LOUKOVITIS et al. (2022). Numerous improvement projects have resulted from the cross-breeding of the Hungarian Merino with other Merinos and breeds descended from Merinos since then. However, due to their historical importance, the indigenous Tsigai of Hungary are now part of a national gene conservation initiative that seeks to preserve their distinctive traits, particularly those that allowed them to thrive in their original Hungarian habitat. However, there has been a recent drop in the Hungarian Merino population, which may be attributable to the fact that Hungarian farmers are increasingly favouring alternative breeds.

To identify pathogens, the innate immune system uses TLRs. Both a strengthening and a weakening of the immune system are possible responses to stress. When the body detects endogenous ligands, such as HSPs, the TLRs in the immune system respond by launching an initial response. Based on their ability to activate the TLR2 and TLR4

pathways, researchers have established that HSPs can be used in research (BEG, 2002). The ability of HSPs to bind to TLRs enhances their ability to present antigens. When these HSPs come into contact with these TLRs, it is possible that dendritic cells and macrophages are activated, and immune-enhancing cytokines are generated. These cytokines are crucial for the host's ability to survive an infection (GOBERT et al., 2003; PAUL et al., 2015). In order to improve thermo-tolerance, ZHAO et al. (2006) discovered that increasing the expression and signalling of *TLRs 2, 4*, and *8* in immune cells can enhance the innate immune response to PAMPs.

This result shows that relative gene expression of *TLR2* and *TLR8* is lowest in the spring and fall and highest in the winter and summer. All breeds showed an increase in expression of both genes over the summer, with the mean relative gene expression value of 12.053±11.018, 3.317±2.720, and 14.263±13.417 for *TLR2* in Hungarian Tsigai, Hungarian Merino, and White Dorper, respectively. While for *TLR8* were 5.747±5.481, 7.497±6.507, and 6.910±8.130, respectively. This is demonstrated also by what BHARATI et al. (2016) found in Thapakar cattle, PAUL et al. (2015) in Bengal goats, VANDANA et al. (2018) in Malabari goats, that *TLR* genes were overexpressed in summers.

Aside from that, the summer was when the highest expression of *TLR4* was shown in Hungarian Merino (15.204±19.950) and White Dorper (14.263±13.417), whereas in the winter, it was seen in Hungarian Tsigai (2.283±1.817). Spring and autumn, which are thermoneutral seasons, still showed a reduced expression. The Hungarian indigenous Tsigai was great at maintaining its immunological response in spite of hyperthermic conditions; this was demonstrated by the fact that this breed has lower *TLR4* expression than other breeds and that this expression was downregulated during the summer. Research conducted by GAVOJDIAN et al. (2015b) indicates that the indigenous Tsigai of Hungary had a lower incidence of lameness and pneumonia compared to the White Dorper and Dorper. BHARATI et al. (2016) highlighted that *TLR4* may play a pivotal role in the immunological response that provides thermo-tolerance and mitigates the negative consequences of prolonged heat stress (inflammation and tissue injury). Additionally, it can detect the lipopolysaccharide (LPS) that is unique to gram-negative bacteria (BULGARI et al., 2017).

The study found that the gene expression of all GOI varied significantly ($p<0.05$) (Appendix Table S5), throughout the year, indicating that gene expression is dynamic and

subject to seasonal change. The seasonal changes in gene expression were not equal in each breed and sex group (Appendix Table S6), suggesting that there was an interaction between breed, sex, and the season in *IL10* gene expression ($p<0.05$). Gene expression levels for the *IL10* and *TLR4* genes varied significantly ($p< 0.05$) across breed groups and seasons, as revealed by the between-group test. Seasonal dynamics revealed that Hungarian Merino sheep were subject to heat stress during summer (as indicated by their relative expression of *HSP70*), but maintained a consistently high level of *IL10* and *TLR2* gene expression, indicating that they maintained an excellent immune response throughout the year. The native Tsigai specimens of Hungary showed remarkable resilience to weather conditions and continues to withstand seasonal stressors, in contrast to the immigrant White Dorper, which was only mildly adapted to the Hungarian climate. Our research on immunological genes and heat tolerance demonstrated that these three breeds are most suited to the environment in Hungary, which is important because of threats posed by both current and future climate change.

4.3. Gene expression study in white-coated and black-coated sheep using RNA-Sequence method

4.3.1. Aligned RNA seq data

This work aims to examine the skin transcriptome generated by RNA-Seq analysis of black-coated and white-coated Hortobágyi Racka sheep (*Ovis aries strepsiceros hungaricus*). CIANI et al. (2020) claimed that this breed is a unique Hungarian national breed, renowned for its morphological trait of spiral V-shaped horns, is frequently utilized in crossbreeding due to its durability and adaptability. The breed has two predominant coat colour phenotypes: completely white and completely black, rendering it very appropriate for the investigation of coat pigmentation. ZSOLNAI et al. (2020) indicate that the two phenotypes of this breed exhibit genetic variations in stress response or adaptation, as seen by the SNPs under selection found in *HTR5A*, according to their 50K SNP analysis. Their findings further suggest that Hortobágyi Racka color variants are genetically distinct breeds although in current nomenclature, the coat colors are considered as just breed variants.

A total of 5,525,285 (approximately 613,921 reads per sample) reads were generated from the Illumina NovaSeq 6000 platform sequencer. The reads were successfully annotated to 21,328 genes from the *Ovies aries* genome (ARS-UI_Ramb v30). The mapping percentages varied between samples (Table 10).

Table 10. The summary of alignment statistics of black (n=4) and white (n=5) coated Hortobágyi Racka sheep

Sample ID	Coat color	Total reads (TPM)	Uniquely mapped	% uniquely mapped	Multiply mapped	% multiply mapped
10.5	White	7164854	4491865	62.69%	2580968	36.02%
10.14	White	6460274	4140255	64.09%	2218596	34.34%
10.13	White	4241093	2144268	50.56%	2026868	47.79%
10.10	White	5393951	3094769	57.37%	2243251	41.59%
10.3	White	7406806	4399639	59.40%	2931958	39.58%
5.1	Black	5478963	2937849	53.62%	2421147	44.19%
5.12	Black	6209220	3770964	60.73%	2377245	38.29%
11.1	Black	4085081	2312890	56.62%	1702935	41.69%
11.8	Black	7577645	5155140	68.03%	2344402	30.94%

TPM: total read per million

4.3.2. Differentially expressed genes between black coated and white coated Hortobágyi Racka sheep

Based on the specifications of FC>1.5 and p-value<0.05, a total of 108 genes (Appendix Table S7) demonstrated differential expression of black coated in comparison to white coated sheep (Figure 13).

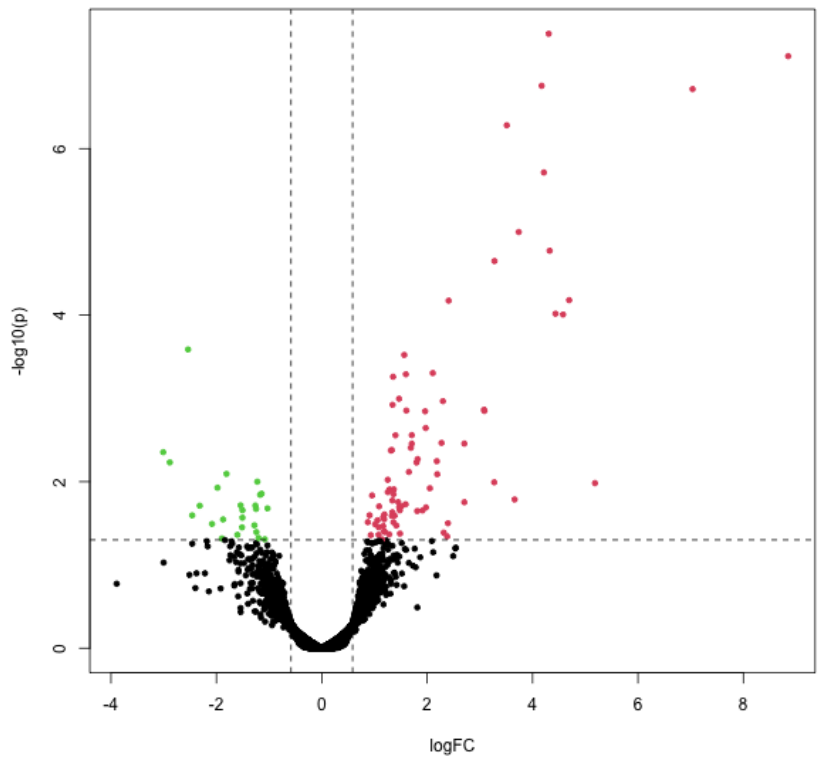

Figure 13. A volcano plot displaying the relationship between log fold change (FC) and log counts per million (CPM).

The green dots and left from dashed lines represent genes that were significantly downregulated, while the red dots above and right from dashed lines represent genes that were significantly upregulated in the comparison of the skin of black and white Hortobágyi Racka sheep. The parameters set are FC>1.5 and P-value<0.05.

Among these, 25 genes were identified as downregulated, whereas 83 genes were classified as upregulated (Figure 14). The quantity of differentially expressed genes (DEGs) in our investigation is less than that reported in the prior skin transcriptomics study involving Boer and Macheng black crossbred goats (XIONG et al., 2019) and Bashibai, Yemule white, and Tulufan black sheep (YAO et al., 2019), yet slightly exceeds the findings in Laiwu Black goat and Lubei White goat (PENG et al., 2018).

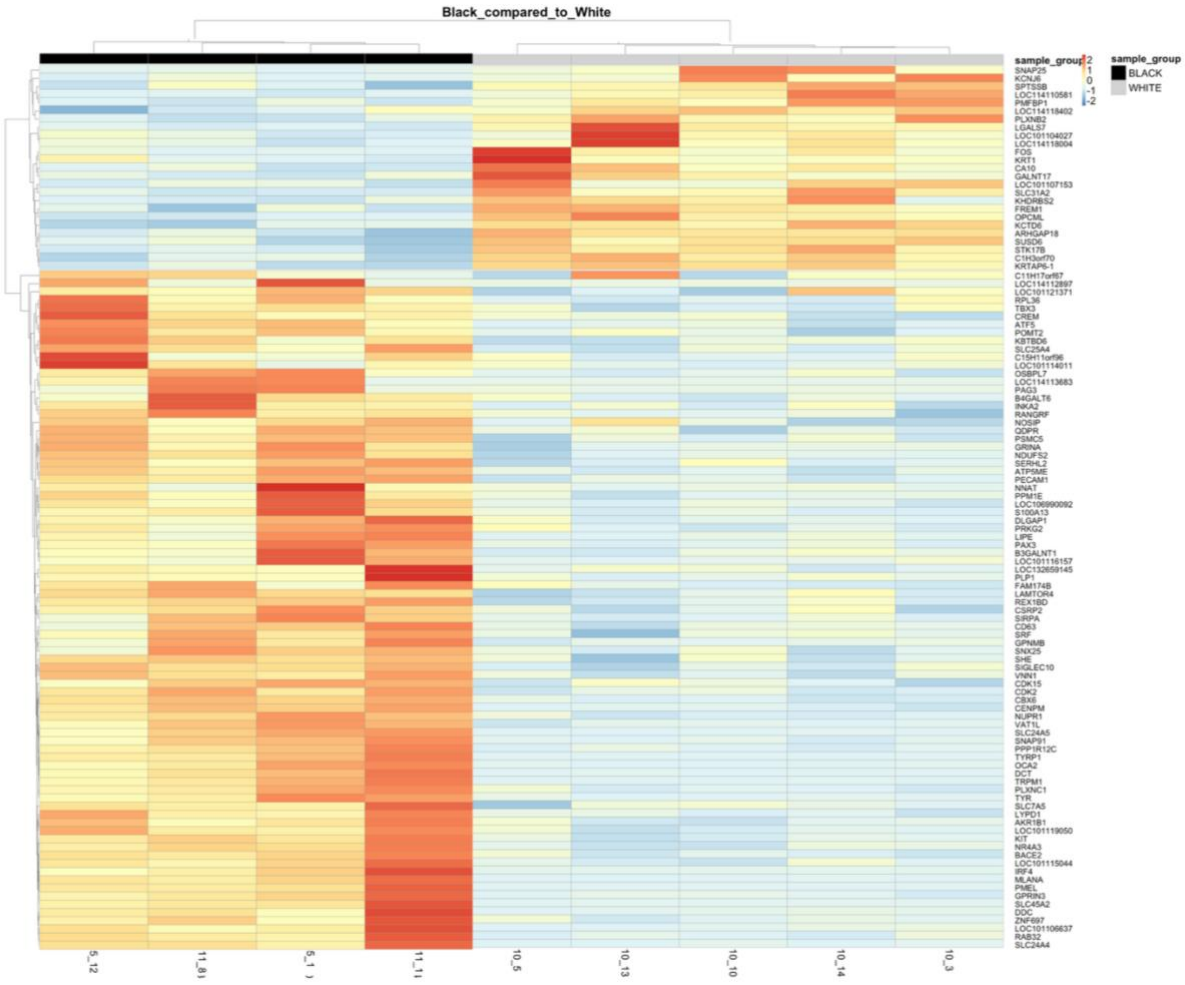

Figure 14. The heat map of the total read per million (TPM) value of 108 differentially expressed genes between the black (n=4) and white (n=5) coated Hortobágyi Racka sheep's skin.

Genes with increased expression are shown in red, while those with decreased expression are shown in blue. The color intensity shows the expression degree.

The result indicated that the quantity of upregulated genes (n= 83) exceeds that of downregulated genes (n=25) when comparing black-coated to white-coated Hortobágyi Racka sheep. The black coat transcriptome was characterized by the overexpression of genes related to pigmentation and melanogenesis, with log fold changes ranging from 3.28 to 8.85 and an adjusted p-value of less than 0.05. This gene set has been identified in previous pigmentation studies of Bashibai sheep, Yemule white sheep, Tulufan black sheep (YAO et al., 2019), Minxian black sheep (SHI et al., 2021), and Bayinbuluke sheep (ZHANG et al., 2023), which indicate a correlation between reduced expression of melanin-related genes and lighter skin/hair phenotypes.

The most significantly downregulated gene was keratin associated protein 6-1 (*KRTAP6-1*) (adj.P.Val=0.192), while the most significantly upregulated genes (adj.P.Val<0.05) included tyrosinase related protein 1 (*TYRP1*), premelanosome protein (*PMEL*), transient receptor potential cation channel subfamily M member 1 (*TRPM1*), melan-A (*MLANA*), solute carrier family 24 member 5 (*SLC24A5*), solute carrier family 24 member 4 (*SLC24A4*), solute carrier family 45 member 2 (*SLC45A2*), tyrosinase (*TYR*), interferon regulatory factor 4 (*IRF4*), dopachrome tautomerase (*DCT*), OCA2 melanosomal transmembrane protein (*OCA2*), and dopa decarboxylase (*DDC*) (Table 11).

Table 11. List of the 12 most significantly upregulated DEGs (adj.P.Val<0.05) and one the most downregulated gene in black coated and white coated Hortobágyi Racka sheep.

Gene ID	Mean black coated (TPM)	Mean white coated (TPM)	logFC	P.Value	adj.P.Val	Up/down regulated
TYRP1	519.976255	0.38323867	8.85035668	7.7507E-08	0.00040381	up
PMEL	430.14116	2.96412931	7.03627363	1.9285E-07	0.00050236	up
TRPM1	659.881621	31.3329627	4.30584532	4.1735E-08	0.00040381	up
MLANA	276.711167	15.08683	4.17133013	1.7604E-07	0.00050236	up
SLC24A5	121.904687	4.00788043	4.6939369	6.6083E-05	0.06350982	up
SLC24A4	31.5492416	0.77828139	4.32397052	1.6827E-05	0.02191697	up
SLC45A2	169.873821	7.62210392	4.21494814	1.9336E-06	0.00335807	up
TYR	93.1604306	3.9810151	4.57812418	9.8362E-05	0.07884073	up
IRF4	23.9985145	0	4.43541439	9.6242E-05	0.07884073	up
DCT	110.914476	7.56152484	3.73708598	1.0038E-05	0.01494242	up
OCA2	401.514072	32.7729986	3.51086441	5.2435E-07	0.00109275	up
DDC	127.383506	11.3946704	3.27680226	2.2394E-05	0.025927	up
KRTAP6-1	7.81475138	46.6352445	-2.5378016	0.00025844	0.19235039	down

TPM: total read per million

4.3.3. Enrichment and pathway analysis

The 108 DEGs between black coated and white coated Hortobágyi Racka sheep were highly enriched in 28 functional annotations, while the 83 upregulated DEGs were significantly enriched in 18 functional annotations, and the 25 downregulated DEGs were

significantly enriched in 2 functional annotations. All are categorized as either CC, BP, or MF.

The combination of upregulated and downregulated DEGs yields 18 significant functional annotations (P.Val<0.05) from the DAVID website. Upon independent analysis of the upregulated (n=83) and downregulated (n=25) genes, 16 significant functional annotations (P.Val<0.05) were identified (Table 12). The combined analysis result omitted GO:0031424~keratinization (GO BP), while the separate up/downregulated DEGs analysis excluded GO:0016310~phosphorylation (GO BP), GO:0031902~late endosome membrane (GO CC) and GO:0032993~protein-DNA complex (GO CC).

Table 12. The significant (P.Val<0.05) enriched GO functional annotations from all, upregulated, and downregulated DEGs between black coated and white coated Hortobágyi Racka sheep

GO category	GO term	*P.Val*		
		All DEGs	Upregulated DEGs	Downregulated DEGs
BP	melanin biosynthetic process from tyrosine	0.0000565	0.000033	
	phosphorylation	0.0385925		
	central nervous system myelination	0.0216751	0.0166092	
	melanocyte differentiation	0.0019120	0.0011240	
	melanosome organization	0.0344566	0.0264441	
	lysosomal lumen pH elevation	0.0087264	0.0066766	
	melanin biosynthetic process	0.0000016	0.0000007	
	developmental pigmentation	0.0259541	0.0198983	
	positive regulation of proteasomal protein catabolic process	0.0216751	0.0166092	
CC	trans-Golgi network	0.0022555	0.0092544	
	intermediate filament	0.0355367		0.0016518
	keratinization			0.0449729
	late endosome membrane	0.0498307		

GO category	GO term	*P.Val*		
		All DEGs	Upregulated DEGs	Downregulated DEGs
	protein-DNA complex	0.0427422		
	melanosome membrane	0.0000000	0.0000000	
	melanosome	0.0000247	0.0000095	
MF	calcium channel activity	0.0092521	0.0059766	
	calcium, potassium: sodium antiporter activity	0.0211729	0.0169171	
	oxidoreductase activity	0.0076164	0.0040589	

GO, gene ontology; BP, biological process; CC, cellular component; MF, molecular function; the grey column means data is not available.

Similar differences were also seen in KEGG pathways analysis; the pathway analysis indicated six highly enriched pathways when all DEGs were evaluated (Figure 15), including oxidative phosphorylation, tyrosine metabolism, metabolic pathways, thermogenesis, melanogenesis and Alzheimer's disease.

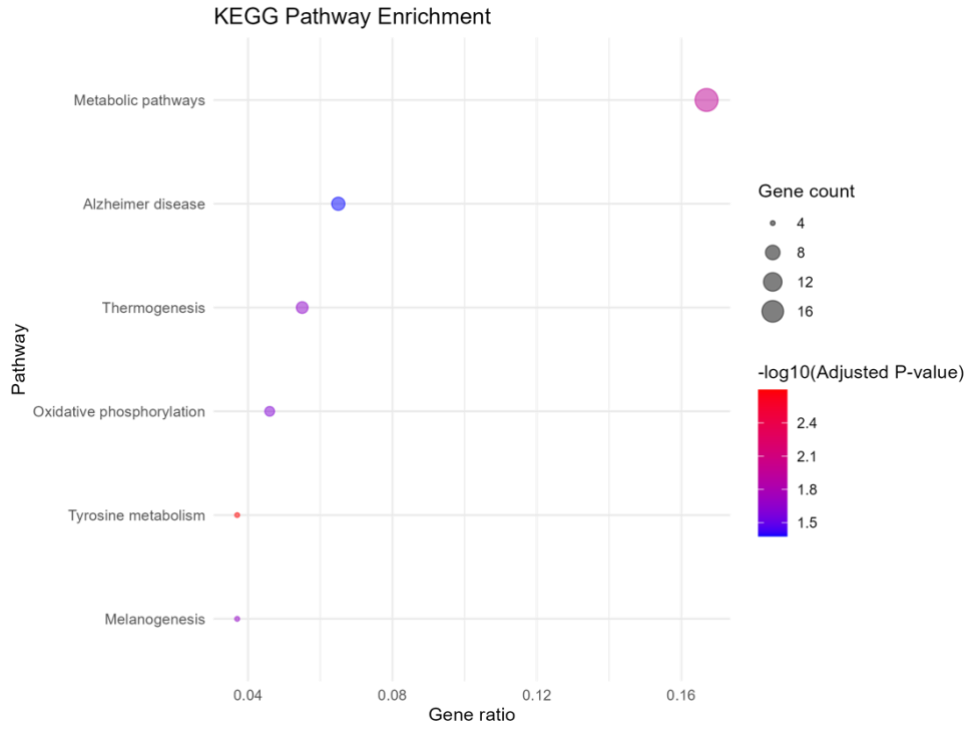

Figure 15. The bubble plot for the KEGG pathway enrichment from the 108 DEGs between black coated and white coated Hortobágyi Racka sheep

Simultaneously, the separation of upregulated DEGs results in the absence of the oxidative phosphorylation and Alzheimer's disease pathways, while the downregulated DEGs did not show any significant enriched pathways (Table 13).

Table 13. Top enriched KEGG pathways (P.Val<0.05) from all and upregulated DEGs between black coated and white coated Hortobágyi Racka sheep. The downregulated DEGs did not show any significant enriched pathways.

Enriched KEGG pathways	*P.Val*	
	Upregulated	Combined
oas00190:Oxidative phosphorylation		0.0249968
oas00350:Tyrosine metabolism	0.0009523	0.0017085
oas01100:Metabolic pathways	0.0193145	0.0112912
oas04714:Thermogenesis	0.0427792	0.0224882
oas04916:Melanogenesis	0.0123643	0.0211667
oas05010:Alzheimer disease		0.0415476

The grey column means data is not available.

Additionally, the STRING database was used to visualize the protein-protein interactions (PPI) of the up-regulated DEGs in the black coated Hortobágyi Racka sheep. Figure 16 shows the interaction network of pigmentation and melanin synthesis (*IRF4, PAX3, SLC24A4, DCT, KIT, TRPM1, MLANA, OCA2, SLC24A5, SLC45A2, TYRP1*, and *TYR*) and immune response and inflammation (*PECAM1* and *GPNMB*) proteins.

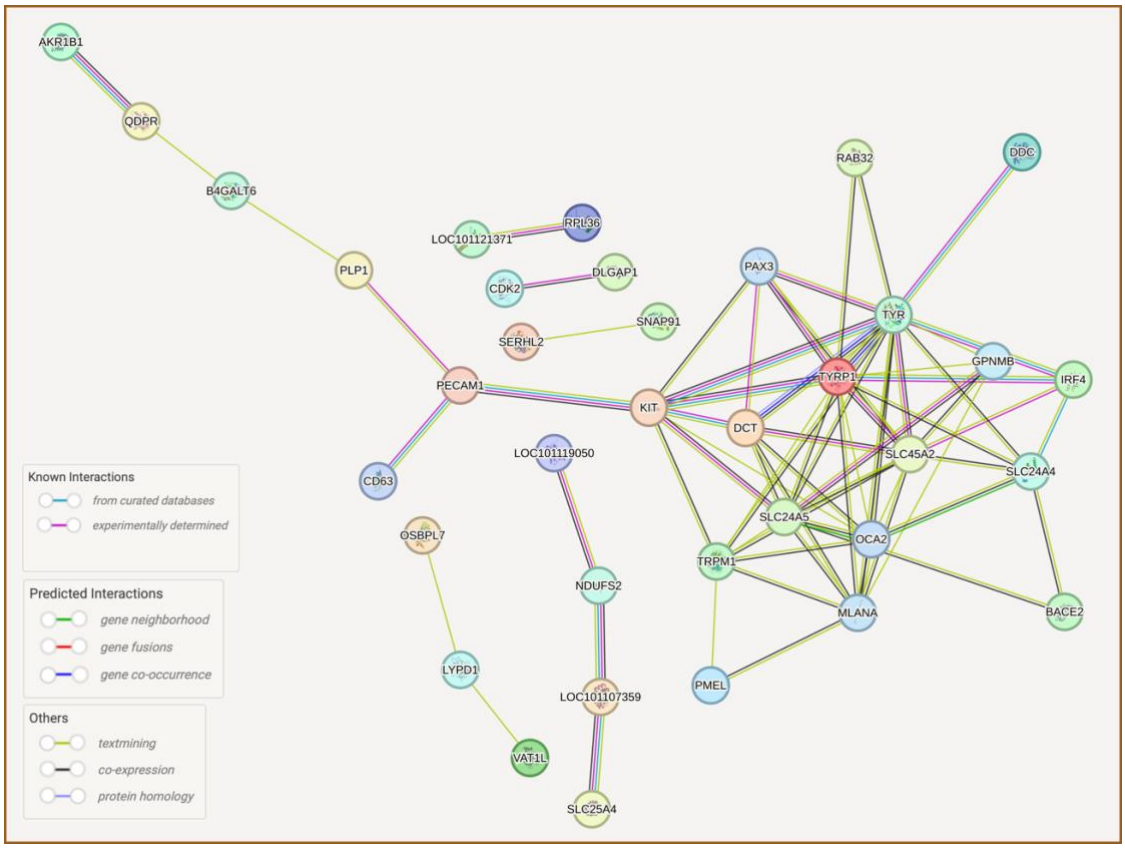

Figure 16. Database search utilizing the STRING database for gene interaction networks using upregulated DEGs.

Each gene is represented as a node, and the connections between them are represented by edges. To improve the display, we eliminate the nodes that are not part of the network. The sort of interaction is indicated by the color of the edge.

TYRP1 is one of three tyrosinase-like enzymes involved in eumelanin production within melanosomes, whereas *PMEL* is responsible for its deposition. *PMEL* and other genes regulate the melanin synthesis pathway, shifting it from pheomelanin to eumelanin (ABDELNOUR et al., 2018; PARIS et al., 2019). *TRPM1* plays a role in melanin transport, encoding a cation channel critical for proper melanocyte function and melanin production (PENG et al., 2015). The transport of sodium, potassium, and calcium is encoded by *SLC24A5* and *SLC24A4*, which regulate melanin production through the maintenance of calcium homeostasis in melanocytes. *SLC45A2* is involved in the transport of molecules across cell membranes, influencing melanin synthesis and transport in melanocytes (DENG et al., 2009; XIONG et al., 2019).

MLANA, in conjunction with *PMEL*, contributes to melanocyte function and the structural stability of key pigmentation enzymes. In this process, tyrosine serves as a key enzyme, with its metabolism regulated by the *TYR* gene, which initiates melanin

production (YAO et al., 2019). The transport of tyrosine is regulated by *OCA2* (WIRIYASERMKUL et al., 2020), with its activation cooperating with the *IRF4* gene (PRAETORIUS et al., 2013). In conjunction with *DCT*, they play a vital role in assessing the quality and quantity of melanin production (VASU et al., 2023). The function of *DDC* in ruminant coat pigmentation remains ambiguous; however, it is essential in the synthesis of melanin precursors, dopa and dopamine, in insects (WANG et al., 2022).

In addition to their roles in pigmentation, the upregulated genes also participate in thermoregulation, stress adaptation, and immunity in livestock. WANG et al. (2020) investigated the liver transcriptome of Dorper sheep and identified that *TRPM1* may facilitate DNA repair and enhance protein stability by increasing calcium ion influx, thereby contributing positively to heat stress response. ALI et al. (2023) indicate that the *TYRP1* gene in animals regulates oxidative stress, facilitates cellular proliferation, and promotes adaptation to extreme conditions. Comparative studies revealed that mutations in *TYRP1* alter protein structure and function, aiding adaptation in native sheep populations. Mutations conserved among various mammalian species influence enzyme function and melanin biosynthesis pathways, thereby impacting survival in diverse environments. *SLC* genes play a crucial role in maintaining central nervous system homeostasis by facilitating the selective and regulated transport of nutrients, hormones, electrolytes, metal ions, and metabolic wastes. This gene family regulates mTOR, which plays a role in protein synthesis and amino acid absorption, and is linked to pro-inflammatory factors (GAOWA et al., 2021; LI et al., 2024b).

This study identified increased expression of *GPNMB* and *PECAM1* in black coated animals. Although not statistically significant, PPI analysis indicated interactions with other overexpressed genes associated with pigmentation. LERTKIATMONGKOL et al. (2016) indicate that platelets, certain immune system cells, and endothelial cells consistently express this gene. It is associated with several important biological processes, including macrophage phagocytosis and the infiltration of inflammatory cells. This gene may interact with β-catenin to regulate the proliferation of endothelial cells and fibroblasts during wound healing in horses (MIRAGLIOTTA et al., 2008). During melanosome development, the type I transmembrane glycoprotein *GPNMB* is present and has an unspecified role in innate immunity (SAADE et al., 2021). LI et al. (2023) reported that *GPNMB* enhances melanin deposition in chicken feathers. The response of ovine microglia to prion infections may be indirectly influenced by *GPNMB* in sheep, as indicated by MUÑOZ-GUTIÉRREZ et al. (2016).

Skin pigmentation, both its process and function, is correlated with immunity. Although their interaction is complex and context-dependent, it remains not fully understood. KOIKE and YAMASAKI (2020) emphasized that melanocytes release cytokines that influence immunological responses, suggesting their potential role as antigen-presenting cells. Melanocytes serve as target cells for T lymphocytes through the processing and presentation of phagocytosed antigens. Intercellular adhesion molecule 1 (ICAM-1) is expressed, serving as a crucial element for immune cell interactions, alongside Cluster of differentiation 40 (CD40), which is generally found in mature dendritic cells and aids in adaptive immunity. Melanocytes containing higher levels of melanin may more effectively suppress inflammation, attributed to the immunosuppressive properties of Levodopa (l-DOPA) and its derivatives. Melanocytes with reduced melanin levels are more actively involved in cytokine-mediated immune signalling, which may enhance the immune response (FU et al., 2020). This indicates that individuals with lower melanin levels may exhibit immunological characteristics that differ from those with higher melanin levels. Although they may not possess superior immunity, the absence of melanin's immunosuppressive effects could result in distinct or more active immune responses.

Previous research has examined the molecular relationship between pigmentation and hormones related to stress adaptation in livestock. Cortisol serves as the primary mediator of the HPA axis, activated in response to stress. The biochemistry of pigment regulation and cortisol regulation is similar, involving the same hormone and receptor families, particularly proopiomelanocortin (POMC). It is assumed that the availability of POMC for cortisol synthesis may be diminished if a greater proportion is allocated for pigmentation purposes. Conversely, variations in receptor sensitivity, including melanocortin receptors, may influence pigmentation and cortisol response. A reduced cortisol response may be beneficial if it helps prevent immunological suppression and other long-term effects of stress, as suggested by the adaptive trade-offs hypothesis (NEJAD et al., 2016; FERNANDES et al., 2023).

Prostaglandins (PGs), key mediators of inflammation, are strongly associated with significant genes involved in melanin formation, including *TYR, TYRP1, DCT, PMEL, MLANA,* and *SLC45A2*, as demonstrated by gene-metabolite correlation studies in Liangshan sheep conducted by ZHANG et al. (2024). PGs play a crucial role in pigmentation and skin adaptation by regulating melanin production and mediating responses to oxidative stress, inflammation, and damage (SRIKANT et al., 2017).

The wool of Hortobágyi Racka sheep is long and coarse, exhibiting no variation in characteristics across different colours if we look with naked eyes. The study of wool of this breed is quite limited, an old study of RYDER (1974) discovered that there was no change in staple length and diameter of this breed wool between sexes or between black and white sheep. However, in another aspect of wool quality, NAGY (2006) observed substantial differences in transparency and medulla content between the two color variants of Hortobágyi Racka in both sexes. In the black-coated animal, the wool was considerably coarser than that of the rams, whereas in the white variant. In this study, a comparison of black-coated and white-coated sheep revealed that the expression of the *KRTAP6-1* gene was the most downregulated in the black phenotype. Previous studies indicated that this gene encodes wool protein, which is regulated differently in fine and strong wool breeds (LI et al., 2019c). It affects the mean fibre diameter in Super Merino (fine wool) and Small Tail Han (coarse wool) sheep (ZHANG et al., 2017), as well as the mean staple strength in Gansu Alpine (fine wool) sheep (SUN et al., 2024). Our finding suggested that the gene may have functions beyond wool characteristics. LITMAN and STEIN (2023) proposed that it may also promote cell proliferation, contributing to tissue homeostasis by balancing cell division with apoptosis.

Animals with dark coat color may possess enhanced resilience to a warming climate, with wool or hair characteristics and structure also playing a role in their thermoregulation capabilities. Lower pigmentation skin exhibits a higher level of oxidative stress than higher pigmentation skin when subjected to heat stress. Eumelanin, responsible for dark pigmentation, exhibits superior antioxidant properties and is more proficient in UV radiation blocking compared to pheomelanin. Its enhanced production may impede ROS formation and mitigate oxidative damage under thermal stress conditions. Darker pigmented skin may exhibit greater heat tolerance compared to lighter pigmented skin due to this factor. MAIBAM et al. (2017) suggested that most breeds originating from tropical regions exhibit dark coats, as seen in Zebu cattle. Their surroundings promote the expression of color genes in skin cells, leading to enhanced skin protection and decreased oxidative damage in response to environmental heat stress. Another fact, a recent study by WEI et al. (2023) on *PMEL* gene editing in Holstein cattle revealed a significant reduction in the ability of color-diluted calves to absorb heat and light when compared to control calves with darker coats. Light-colored coats may demonstrate lower absorption rates, while dark-colored animals have inherent mechanisms to regulate high heat absorption and mitigate negative effects.

Given that pigmentation is induced by environmental stressors as a natural adaptation mechanism, skin pigmentation may undergo evolutionary changes due to climate change (ALEENA et al., 2018; LEITE et al., 2020; ZHANG et al., 2024).

4.3.4. RNA-seq data validation with RT-qPCR

RT-qPCR was employed to quantify the expression of 10 randomly chosen from DEG list in the skin of black and white Hortobágyi Racka sheep in order to verify the RNA-Seq result. Known genes associated with immunity (*IRF4, PLXCN1, GPNMB*), metabolic pathways (*TRPM1, VNN1, PLP1*) and ruminant skin pigmentation *(TYRP1, SLC24A5, PMEL, TYR*) were among those found to be 108 differently expressed in white and black sheep skin according to transcriptome sequencing result. In line with the transcriptome sequencing data, 8 out of 10 chosen genes exhibited significantly increased expression in black sheep skin compared to white sheep skin, according to real-time PCR results. When comparing the expression of genes in sheep with black and white skin, *TYRP1* displayed the most differential expression (Figure 17).

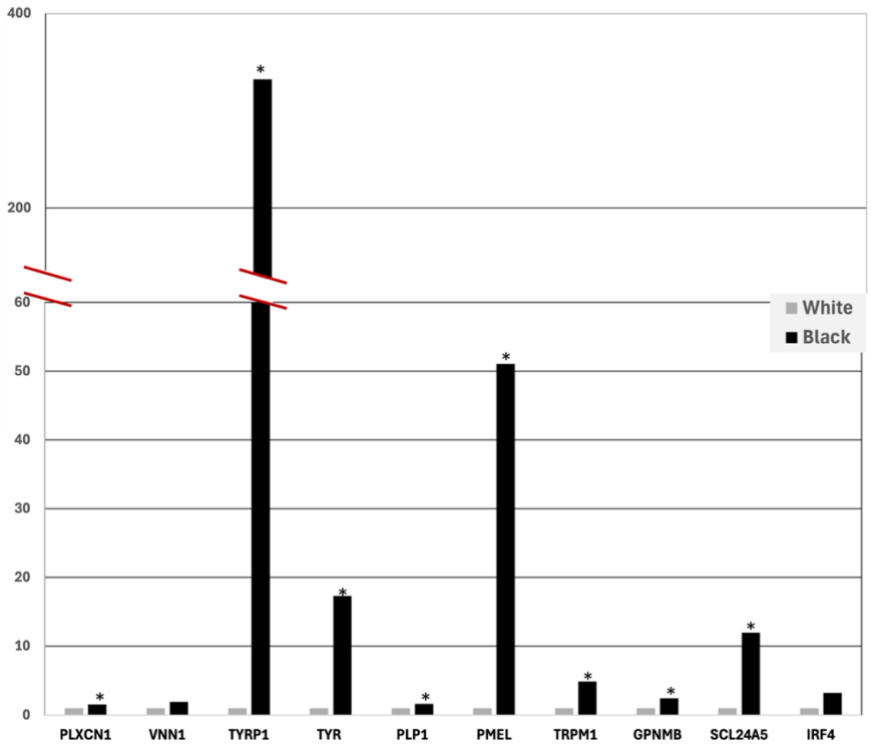

Figure 17. RT-qPCR validation of 10 differentially expressed genes in white and black coated Hortobágyi Racka sheep skin.

The expression level of target genes was normalized relative to that of the GAPDH *gene. The bars in each panel represent the mean and *P < 0.05.*

5. CONCLUSIONS, RECOMMENDATIONS

5.1. Conclusions

As demonstrated in the first part of the research, the KASP-PCR method makes it possible to investigate polymorphisms in different sheep breeds. The allele and genotype frequencies for the *HSPA12A* and *HSPA8* SNPs indicate that these genes may be used as future markers for thermotolerance adaptation in sheep. According to principal component analysis, the most significant possibilities are the *IL33* and *HSP90AA1* SNPs. These results improve our understanding of the genetic diversity of SNPs involved in thermotolerance adaptation in sheep.

The second study found that the relative expression of heat stress and immunity genes, such as *HSP70, IL10, TLR2, TLR4*, and *TLR8*, varies seasonally among the three sheep breeds studied. This provides more evidence that sheep's thermo-balance and immunity are affected by seasonal stressors. Compared to the indigenous Hungarian Tsigai and tropical White Dorper breeds, the Hungarian Merino has a poorer thermotolerance. When it comes to staying resilient under heat stress, nevertheless, the White Dorper is unable to hold an advantage over the Hungarian indigenous Tsigai and, secondarily, the Hungarian Merino.

The third research validated the association between black coat pigmentation in Hortobágyi Racka sheep and the genes *TYRP1, PMEL, TRPM1, MLANA, SLC24A5, SLC24A4, SLC45A2, TYR, IRF4, DCT, OCA2*, and *DDC*. Furthermore, in addition to its potential influence on wool characteristics, our findings suggest a broader role for *KRTAP6-1*. The roles of these genes in adaptation occur through pleiotropic effects. It seems that animals with darker coats possess more effective mechanisms for managing high heat absorption and reducing adverse effects, whereas animals with lighter coats tend to absorb less heat by default. Their black coat may either assist in thermoregulation during hot weather or serve as an adaptive response to heat through their dark skin coat. The question of which came first remains a causality dilemma.

5.2. Recommendations

For a better comparison, it might be more useful to include samples from extremely cold-weather regions (such as Iceland, Finland, and Norway) in the first research phase. Furthermore, further research is required to clarify the connection between the SNPs that were examined and heat resistance in sheep. Further investigation into the observed SNPs'

marker associations and their marker-quantitative trait locus phase relationships in each population is needed to accurately characterize the effects of each SNP.

In the future study for the second phase of the research, it would be beneficial to incorporate additional observed parameters, such as physiological and behavioural assessment (e.g., respiratory rate, skin temperature, rectal temperature, panting behaviours, and pulse rate) or blood biochemistry profiles, during the sample collection days. These measures would be advantageous for verifying the heat stress level, understanding its manifestations, and its relationship to the hormonal mechanisms of adaptation.

It would be interesting to explore the interactions between the biochemical pathways associated with coat and skin colouration and other biochemical pathways, such as keratin synthesis and dopamine, given that the findings from the third phase of the study revealed the complexity and interconnectivity of coat and skin colouration. Also, to clarify the complex mechanism of adaptation, it would be advantageous to incorporate gene expression of other organs that are related to the adaptation mechanism (e.g., brain, liver, fat tail, etc.).

6. NEW SCIENTIFIC RESULTS

1. rs161504783-*HSPA12A,* rs588145625-*HSPA8,* rs397514117-*HSP90AA1,* rs397514272-*HSP90AA1*, and rs410259751-*IL33* are identified as potential candidate SNP markers for thermotolerance adaptation in sheep.
2. Sheep's thermoregulation and immunity are affected by seasonal stressors, and can be observed through the seasonal expression dynamics of *HSP70, IL10, TLR2*, *TLR4*, and *TLR8* genes. Among the three breeds studied, the Hungarian indigenous Tsigai has exceptional thermotolerance and immunity that are suited to the continental climate of Hungary.
3. This study also successfully identified 108 differentially expressed genes by comparing the skin of black- and white-coated Hortobágyi Racka sheep using the RNA-seq method. Additionally, the genes *TYRP1, PMEL, TRPM1, MLANA, SLC24A5, SLC24A4, SLC45A2, TYR, IRF4, DCT, OCA2,* and *DDC* are associated with black coat pigmentation in Hortobágyi Racka sheep. The *KRTAP6-1* gene was found to be a promising factor in sheep immunity beyond merely influencing wool properties. The pigmentation-related genes in sheep may also influence the animal's adaptability to heat stress, albeit through a pleiotropic mechanism. Also, black-coated and white-coated might have a different heat stress adaptation mechanism.

7. PRACTICAL RESULTS

1. These research on genetic diversity demonstrated that our sheep population still have a robust genetic pool that can serve as a foundation, offering potential for ongoing adaptive improvement to ensure climate-resilience livestock production. Especially the indigenous sheep breeds potential despite the increasing popularity of international breeds. By maintaining and improving these breeds, we can secure a more sustainable and resilient livestock sector that advantages both local populations and global biodiversity.
2. The SNP markers identified in this work (rs161504783-*HSPA12A,* rs588145625-*HSPA8,* rs397514117-*HSP90AA1,* rs397514272-*HSP90AA1*, rs410259751-*IL33*) can be implemented into breeding strategies that improve heat and disease tolerance in sheep.
3. Examining how homeothermic sheep are influenced by seasonal stressors and how their morphological features (e.g., coat color) significantly impact their responses necessitates the integration of improved management practices in both housing and breeding to mitigate the adverse effects of climate change. Variations in transcript levels of pigmentation-related genes across animals of different coat colors can elucidate the molecular mechanisms governing pigmentation. It can assist in identifying markers for selective breeding related to coat color, adaptability, immunity, and the promotion of animal welfare in the context of heat stress challenges.

8. SUMMARY

This research aimed to enhance our comprehension of the genetic architecture of heat stress in sheep (*Ovis aries*) through SNPs polymorphism study and transcriptomics study, conducted in three unrelated researches.

The first research investigated the polymorphism of heat resistance-related genes across several sheep breeds from diverse climatic conditions. A study involving 51 SNPs across 30 genes associated with heat stress was conducted on 720 sheep from 17 distinct breeds acclimated to various temperatures in Hungary, Bosnia and Herzegovina, Morocco, and Romania, utilizing KASP-PCR technology. A total of 601 animals (83.47%) out of 720 were successfully genotyped, and 32 SNPs (62.74%) from the initial list of 51 SNPs were successfully genotyped in this study; 17 of these were identified as polymorphic (33.33%), which are the following genes: *HSPA12A, HSP90AA1, IL33, DIO2, BTNL2, CSN2, ABCG1, CSN1S1, GHR, HSPA8, STAT3,* and *HCRT*. All breeds were successful in genotyping for four SNPs: rs161504783-*HSP12A*, rs588145625-*HSPA8*, rs588498137-*STAT3*, and rs602521720-*HCRT*. The two SNPs that showed patterns of allelic and genotypic frequencies in relation to climatic characteristics were *HSPA12A* and *HSPA8*. In contrast to earlier findings, the A allele for the *HSPA8* SNP was absent in heat-tolerant breeds and was solely identified in cold-tolerant breeds. The Botosani Karakul breed exhibits the highest number of SNPs (4 SNPs) deviating from Hardy-Weinberg equilibrium, and has decreased heterozygosity noted in 10 SNPs. A potential explanation for this is that Botosani Karakul, a Romanian crossbreed, has seen significant genetic admixture throughout its breeding history. PCA failed to distinctly discriminate the breeds, however plot concentration exhibited slight variation among the three groups, with the loading values of *HSP90AA1* and *IL33* SNPs strongly influencing PC1 (11.21%) and PC2 (9.98%). All breeds were predominantly clustered within the range of −2.50. We validated prior studies indicating that the SNPs of *HSPA12A, HSPA8, HSP90AA1,* and *IL33* are possible candidate markers for thermotolerance adaption in sheep.

The second research is a gene expression analysis of seasonal variations in heat-related genes across various sheep breeds in Hungary. The expression of genes associated with heat stress and immunity *(HSP70, IL10, TLR2, TLR4*, and *TLR8*) in three prevalent sheep breeds in Hungary — the indigenous Tsigai, Hungarian Merino, and White Dorper — was assessed using qRT-PCR during the peak summer, fall, winter, and spring seasons. The THI on all sampling days remained within the thermoneutral zone, except during the

summer season, which recorded a value of 78.99. The seasonal stressor influenced the relative gene expression of all genes examined in this study. Among all genes of interest in this study, the lowest expression relative to the spring season was observed in the autumn. This study observed that in the Hungarian Merino, a cold-tolerant breed highly susceptible to heat stress, the relative gene expression of *HSP70* was elevated throughout summer, demonstrating heat-stress-induced *HSP70* expression. The *IL10* gene showed an increase in expression during the summer for Hungarian Tsigai, but for Hungarian Merino and White Dorper, the highest expression was identified in the winter season. Spring and autumn are seasons when *TLR2* and *TLR8* gene expression is lower, whereas winter and summer are seasons when relative gene expression is higher. All breeds exhibited overexpression of both genes during the summer. The peak expression of *TLR4* was recorded in the summer for Hungarian Merino (15.204 ± 19.950) and White Dorper (14.263 ± 13.417), but Hungarian indigenous Tsigai exhibited its maximum expression in the winter (2.283 ± 1.817). The indigenous Hungarian Tsigai was clearly the hardiest breed, having developed remarkable thermotolerance and immunity to the harsh Hungarian climate. In addition, Hungarian Merino maintained a good immune system all year round, even though they were heat stressed in the summer.

The third research carried a transcriptome analysis of the skin of Hortobágyi Racka sheep with black (n=4) and white (n=5) coat colors, used high-throughput RNA sequencing, and linked it with thermoregulatory capacity. A total of 5,525,285 reads were sequenced using the Illumina NovaSeq 6000 platform sequencer. The readings were effectively annotated to 21,328 genes from the *Ovis aries* genome (ARS-UI_Ramb v30). Based on the criteria of FC>1.5 and p-value<0.05, a total of 108 genes demonstrated differential expression between black-coated and white-coated sheep. Of these, 25 genes were identified as downregulated, whilst 83 genes were classified as upregulated. The gene exhibiting the greatest downregulation was *KRTAP6*-1 (adj.P.Val= 0.192) which a keratin related gene whereas the most significantly elevated genes (adj.P.Val<0.05) included *TYRP1, PMEL, TRPM1, MLANA, SLC24A5, SLC24A4, SLC45A2, TYR, IRF4, DCT, OCA2,* and *DDC,* which are genes linked to pigmentation and the biological mechanisms of melanogenesis. The GO and KEGG pathway analysis of DEGs indicated that the majority were linked to biological processes and cellular component categories. Our results particularly highlighted processes associated with oxidative phosphorylation, tyrosine metabolism, metabolic pathways, thermogenesis, and melanogenesis. The STRING database was utilized to illustrate the protein-protein interactions, revealing the

interaction network associated with pigmentation and melanin synthesis (*IRF4, PAX3, SLC24A4, DCT, KIT, TRPM1, MLANA, OCA2, SLC24A5, SLC45A2, TYRP1,* and *TYR*) as well as immune response and inflammation (*PECAM1* and *GPNMB*) ribosomal proteins. The findings indicated that the roles of these pigmentation-related genes in heat stress adaptation occur via pleiotropic effects. Animals with darker coats seem to possess superior mechanisms for managing excessive heat absorption and alleviating adverse effects, whereas those with lighter coats inherently absorb less heat.

This dissertation research has contributed to the understanding of the genetic architecture and molecular basis of adaptive traits in sheep, mediated by complicated mechanisms involving large gene networks in both direct and indirect (pleiotropic) ways.

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10. PUBLICATIONS IN THE FIELD OF RESEARCH

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Registry number: DEENK/404/2025.PL
Subject: PhD Publication List

Candidate: Putri Kusuma Astuti
Doctoral School: Doctoral School of Animal Husbandry
MTMT ID: 10079965

List of publications related to the dissertation

Foreign language scientific articles in Hungarian journals (1)

1. **Astuti, P. K.**, Wanjala, G., Bagi, Z., Kusza, S.: Coping with climate change; is white sheep more favorable than black? = Szembenézni az éghajlatváltozással; kedvezőbb a fehér bárány a feketénél? : a review = irodalmi áttekintés.
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In: Biotechnológia a Debreceni Egyetemen 2024 Szimpózium : Absztraktfüzet, Debrecen Egyetem, Debrecen, 49, 2024.

29. Triatmojo, A., **Astuti, P. K.**, Guntoro, B., Latorcai, C., Kusza, S., Strausz, P.: Management manners and organizations supporting the utilization of scientific results of genetics in practice - opportunities and challenges in Europe and Indonesia.
In: Genetics and Applications: An Aspiring Interdisciplinary Journal of Genetic Research, Speciasl edition, Book of abstracts, Institute for Genetic Engineering and Biotechnology, University of Sarajevo, Sarajevo, Bosnia and Herzegovina, 41, 2023, (eISSN 2566-431x) ISBN: 2566431x

30. Wanjala, G., Ilisiu, E., Gavojdian, D., Bagi, Z., **Astuti, P. K.**, Kusza, S.: Genomic diversity and population structure of Hungarian and Romanian native sheep breeds.
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32. Wanjala, G., **Astuti, P. K.**, Kichamu, N., Kusza, S.: Eco-regional influence on genetic diversity, population structure, and signatures of selection of indigenous sheep in Kenya. In: Biotechnológia a Debreceni Egyetemen. Fókuszban az agrár- és élelmiszerbiotechnológia : Tudományos Szimpózium előadás összefoglalók, Debreceni Egyetem MÉK Agrár Genomikai és Biotechnológiai Központ, Debrecen, 46-47, 2021. ISBN: 9789634903512

Total IF of journals (all publications): 44,096
Total IF of journals (publications related to the dissertation): 13,1

The Candidate's publication data submitted to the Tudóstér have been validated by DEENK on the basis of the Journal Citation Report (Impact Factor) database.

17 June, 2025

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11. STATEMENTS

DECLARATIONS

I wrote this thesis in the framework of the University of Debrecen Doctoral School of Animal Science for the purpose of obtaining a doctoral degree (Ph.D.) at the University of Debrecen.

Debrecen, 7 July 2025

Putri Kusuma Astuti
PhD candidate

DECLARATION

I hereby certify that the doctoral candidate **Putri Kusuma Astuti,** has carried out her work under my supervision within the framework of the Doctoral School of Animal Science, University of Debrecen, between 2021-2025 the candidate has made a decisive contribution to the results of the thesis through his/her independent creative work, and the thesis is the candidate's independent work. I recommend that the thesis be accepted.

Debrecen, 7 July 2025

Prof. Dr. Szilvia Kusza, D.Sc.
Supervisor

12. ACKNOWLEDGEMENT

I am particularly grateful of my supervisor, Prof. Szilvia Kusza, who gave me the chance to complete this PhD program and offers priceless and continuous guidance that has been very helpful for me in completing the research and writing my dissertation. I am sincerely grateful for all the trust and opportunities I have been given, which have been invaluable in shaping my journey and enabling me to grow both personally and professionally.

I would want to thank the Doctoral School of Animal Science, University of Debrecen, for allowing me to pursue my education, and the Tempus Public Foundation within the Stipendium Hungaricum Program for granting me the scholarships. My heartfelt thanks also go to Dr. Oláh János, Dr. Zoltán Bagi, Dr. Bianka Véghné Tóth, Dr. George Wanjala, Katalin Balog, Bettina Hegedűs, and Nelly Kichamu, whose camaraderie have turned the challenges of being far from home and balancing work into a manageable experience.

I extend my heartfelt thanks to all collaborators, including Roland Fajardo, Peter Sárkány, Dr. Lilla Bodrogi, Dr. Tímea Pintér, Dr. Gabriella Skoda and our international partners, for their invaluable support in sample procurement, as well as the partner laboratories, farms, and slaughterhouse that played a crucial role in facilitating this research. I am also deeply grateful to my home institution, Faculty of Animal Science, Universitas Gadjah Mada, for granting me the opportunity to take study leave and pursue this endeavour.

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13. ANNEXES

Appendix Table S1. Primers of the 51 SNPs set.

SNP	Locus	Gene name	Allele substitution	Chromosome	Sequence
rs593507294	*LEP*	Leptin	C/T	4	ACCAGGATCAATGACATCTCACACA[C/T]GGTAGGGAAGGACAGGGAGATGAGG
rs161110765	*SOCS3*	Suppressor of cytokine signaling 3	A/C	11	GCCGCCGGCGCCCCCTCGTTCTCCC[A/C]GCCCCCCGCTGAACCCTCCTCCTCG
rs161286575	*PPARG*	Peroxisome proliferator-activated receptor gamma	C/T	19	AACGGAATGTCCTCATAGTGCGGAG[C/T]GGAAATGCTGGAGAAGTCAACGGTG
rs603870279	*ASIP*	Agouti signaling protein	C/T	13	TCCAAAAAGATCAGCAGAAATGAAG[C/T]GGAAAAGAAGAAAAGAGCTTCCAAG
rs598380853	*ASIP*	Agouti signaling protein	C/G	13	GGCGTTTCCCCACAGAGAAAGGCTC[C/G]GATGAAGAACGTGGCACGGACCCGG
rs601650611	*ASIP*	Agouti signaling protein	C/G	13	CGGACCCGGCCCCCGCCGCCTACCC[C/G]CTGCGTGGCCACCCGCGACAGCTGC
rs420959261	*CSN1S1*	Casein alpha s1	C/T	6	ATTGGCTCTGAGAACAGTGGAAAGA[C/T]TACTATGCCACTGTGGTGGTAAGTT
rs587905107	*CSN1S1*	Casein alpha s2	C/T	6	AAACTTCTCATCCTTACCTGTCTTG[C/T]GGCTGTTGCTCTTGCCAGGCCTGTG
rs416941267	*CSN2*	Casein beta	G/T	6	GGGAAGGGTCCCCGGACAGGACCAA[G/T]TACAGGCTCCTGGTACAGCAGAAAG
rs430298704	*CSN2*	Casein beta	C/T	6	TACAGCAGAAAGGCCTGGATGGGCA[C/T]ATCTCTCTGGGGCACTGCTTTCTGG
rs420611298	*ABCG1*	ATP binding cassette subfamily G member 1	G/T	1	CTGGGAGGGCAAGTGCGACTCTGAC[G/T]GCAGGAGAGAGCCTGAGGGCGATGC
rs159956881	*ABCG2*	ATP binding cassette subfamily G member 1	A/G	6	GAATTAGATAAATTCTCAGGGGAGC[A/G]GAGAAGGAAGAAGCTTTCATCCTAT
rs159876394	*IGF1*	Insulin like growth factor 1	C/G	3	CTCGGGTCCCGCCGTGGCAGAGCTG[C/G]TGAAGGCGAGCAAGCACAGGGCCAG
rs160257833	*ESR1*	Estrogen receptor 1	A/G	8	GGTGCCCTCCGGAGACATGAGAGCT[A/G]CCAACCTTTGGCCAAGCCCCATCAT
rs591182158	*ESR1*	Estrogen receptor 1	A/G	8	GGAAGAGAAGGACCACATCCACCGC[A/G]TCCTGGACAAGATCACAGACACCTT
rs598908205	*GNRH1*	Gonadotropin releasing hormone 1	C/T	2	GGTCGATCAGCCAGTAGAACCTAAG[C/T]ACTGTGGGTGCATTGTTCACCAGTC
rs411181557	*DIO2*	Deiodinase iodothyronine type II	C/G	7	TCGAAAAACAAAGAAGAATCCCCAG[C/G]CACCGCCCAACCATCTGAAGGATGA
rs414917134	*BTNL2*	Butyrophilin like 2	C/G	20	AGGTGGTACCGCTTGGAGCCCAGCA[C/G]TCCTGTGTTTTTGTATCGGGATGGA
rs405270595	*BTN1A1*	Butyrophilin	A/G	20	GTCCTCCCCCATGGGGGACAGTGGG[A/G]TCTCCTTTGAAATGTCCTTGGCATC
rs161146164	*GHR*	Growth hormone receptor	G/T	16	ACAGGTATCTCAGAACTTGGAACAT[G/T]TTCTGCTGTCCCTGACCTCCCGGCT
rs55631463	*GHR*	Growth hormone receptor	A/G	16	AGAAGTGGTCACACCCAGCCAAGCA[A/G]ACTTCATCGTGGACAGCGCTTACTT
rs407318935	*STAT1*	Signal transducer and activator of transcription 1	A/G	2	AACGGATGGTGGCAAATGAAACATC[A/G]TTGGCAGCATGCTCCCTAGGAGGGT
rs161691559	*HSP90AB1*	Heat shock protein 90 alpha family class B member 1	A/G	20	ATCCATGAGGACTCCACTAACCGGC[A/G]ATGCCTTTCTGAGCTGCTGCGCTAT
rs397514115	*HSP90AA1*	Heat shock protein 90 alpha family class A member 1	-/C	18	CTAGACCCTCTAATCGCTGCCCGGA[-/C]AAACCCCTAACCATGGACCTGAGCC
rs397514116	*HSP90AA1*	Heat shock protein 90 alpha family class A member 1	C/G	18	CCTCTAATCGCTGCCCGGACCCCCC[C/G]AAACCCCTAACCATGGACCTGAGCC
rs397514117	*HSP90AA1*	Heat shock protein 90 alpha family class A member 1	A/C	18	ATGCCCCAAGCATATACCTGTGACA[A/C]GGGACTGGACCCCTCCCCCGGCTTT
rs397514269	*HSP90AA1*	Heat shock protein 90 alpha family class A member 1	A/G	18	AGGCTCGAAACCTAGCCCCAGGCCC[A/G]TAGTCGGGGGGTCCCGATGCTGGGG
rs397514270	*HSP90AA1*	Heat shock protein 90 alpha family class A member 1	G/T	18	TCGAAACCTAGCCCCAGGCCCATAG[G/T]CGGGGGGTCCCGATGCTGGGGGGAT
rs397514271	*HSP90AA1*	Heat shock protein 90 alpha family class A member 1	A/G	18	GAAACCTAGCCCCAGGCCCATAGTC[A/G]GGGGGTCCCGATGCTGGGGGGATCC
rs397514268	*HSP90AA1*	Heat shock protein 90 alpha family class A member 1	-/G	18	TAGCCCCAGGCCCATAGTCGGGGGG[-/G]TCCCGATGCTGGGGGGATCCGGAGC
rs397514272	*HSP90AA1*	Heat shock protein 90 alpha family class A member 1	G/T	18	GCGCACACTATCTGGGGACACGCTT[G/T]AACCACCGAGAGTTTACTTTTCTGG
rs397514273	*HSP90AA1*	Heat shock protein 90 alpha family class A member 1	A/G	18	TTAACCACCGAGAGTTTACTTTTCT[A/G]GACTTCCCAGAGCTGCTGGCACGGG
rs588145625	*HSPA8*	Heat shock protein family A (HSP70) member 8	A/G	15	AACCTCTTCAATGGTGGGCCCAGAG[A/G]AGGCACCACCTGATGGAGGAGCTCC
rs161504783	*HSPA12A*	Heat shock protein family A (HSP70) member 12A	C/T	22	GTCCGACTCTGCCGATTACGTCGTA[C/T]GTGTTCCTTAGCTAGTGGCCAACAC
rs160077209	*HSPA4*	Heat shock protein family A (HSP70) member 4	A/G	5	TGCTGACATGCTGAAAGTGGAAAAA[A/G]GCACGAATGAGGCCATGGAGTGGAT
rs589164764	*IL1R1*	Interleukin 1 receptor type 1	C/T	3	CTTCCAGTAGACATAGTTACTTAGC[C/T]GGCCAGTAACATTGCAGATCAATTG
rs160387232	*IL1R1*	Interleukin 1 receptor type 1	C/T	3	CAGGAGGCTGATTATCATTTTGGTC[C/T]CAGAAACGTCAGGATTCAGCTGGCC
rs590620426	*IL2*	Interleukin 2	C/G	17	CAACTCTTGTCTTGCATTGCACTAA[C/G]TCTTGCACTCGTTGCAAACGGTGCA

rs596312311	*IL2*	Interleukin 2	C/T	17	AACGGTGCACCTACTTCAAGCTCTA[C/T]GGGGAACACAATGAAAGAAGTGAAG
rs416425182	*TR*	Thyroglobulin	A/C	9	GCTCACCGCGTGGCCTGCTCCAGGG[A/C]GCGGGAGAAGGAGGCGTAGTCGTCT
rs595200178	*TR*	Thyroglobulin	A/G	9	CTGAGCCCTCTCTGGCCTGATGACC[A/G]CAGCAGGGGAAAGCGCAGAGCCACC
rs418400798	*TR*	Thyroglobulin	C/T	9	GGCTTCCACGAAGTGCCCACTTGAA[C/T]GGCCTGCGACCTGCCCAGCAGCTGG
rs410259751	*IL_33*	Interleukin 33	G/T	2	CCGTTACTATGAATCCCAATTCCCC[G/T]TAAGTGAAACAGGTAATTTGGGAGG
rs162295351	*HSP90AB1*	Heat shock protein 90 alpha family class B member 1	A/C	20	CTTGGACAAGATTCGCTATGAGAGC[A/C]TGACAGACCCTTCCAAATTGGACAG
rs161691552	*HSP90AB1*	Heat shock protein 90 alpha family class B member 1	A/G	20	GAACCCTGATGACATCACCCAGGAG[A/G]AATATGGAGAGTTCTACAAGAGTCT
rs597293577	*STAT_PIAS3*	Protein inhibitor of activated STAT 3	C/T	1	AACCTTCTTCTTACTCTCCGATGGA[C/T]TGCCCTCTTGGACTGGGCTATACTG
rs593155540	*STAT_PIAS3*	Protein inhibitor of activated STAT 3	A/G	1	ACTCGTAGTAGCCACCTCACTGTCC[A/G]GGTCAGCAGTCAACTTCTCCTTGAC
rs602521720	*HCRT*	Hypocretin neuropeptide precursor	C/G	11	CCGCGCGAGAGCTCAGACCCCGGAC[C/G]GTCCCCCAGGTGCCACGGACGAGGC
rs425706327	*USP19*	Ubiquitin specific peptidase 19	A/G	19	CGGCATTTCTTCTGAGATGGTGGCC[A/G]GTGGGCCCATTGAAGTTGGCGCCTT
rs161274296	*USP19*	Ubiquitin specific peptidase 19	G/T	19	ACGTGTTCTATCCTCTGGTATCCCA[G/T]AGCCCTTGGAGATGAGCTCACCGGC
rs588498137	*STAT3*	Signal transducer and activator of transcription 3	A/G	11	GCTTTACCTGGACACGCTTGCGGAC[A/G]TCCTGCAGGTGCTGCTCCAGCATCT

Appendix Table S2. Polymorphic SNPs and genotype frequencies in the 15 sheep breeds

			POP 1: Pramenka							POP 2: Beni Guil						POP 3: D'Man						
	GENO TYPE	ALLELE	Genot. Freq	Allele Freq	Fis	x^2	H obs	H exp	n	Genot. Freq	Allele Freq	Fis	x^2	H obs	H exp	n	Genot. Freq	Allele Freq	Fis	x^2	H obs	H exp	n
SNP1	CC	C	8	3,689	-0,129	0,413	0,5625	0,502	64	1	0,500	-0,833	15,834*	0,927	0,511	48	0	0,432	-0,760	11,970*	0,8636	0,502	44
	TC	T	18	0,469	-0,129					22	0,500	-0,833					19	0,568	-0,76				
	TT		6							1							3						
SNP2	CC	C	21	0,818	-0,220	1,476	0,354	0,302	66	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	GC	G	12	0,000	-0,220					NA	NA	NA					NA	NA	NA				
	GG		0							NA							NA						
SNP3	AA	A	0	0,000	0,000	X	0	0	54	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	CA	C	0	0,000	0,000					NA	NA	NA					NA	NA	NA				
	CC	C	54	1,000						NA							NA						
SNP4	AA	A	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	GA	G	NA	NA	NA					NA	NA	NA					NA	NA	NA				
	GG		NA							NA							NA						
SNP5	GG	G	0	0,050	-0,526	0,055	0,1	0,0967	60	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	TG	T	3	0,950	-0,526					NA	NA	NA					NA	NA	NA				
	TT		27							NA							NA						

SNP6	**AA**	**A**	0	0,224	-0,289	2,206	0,448	0,381	58	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	GA	**G**	13	0,776	-0,289					NA	NA	NA					NA	NA	NA				
	GG		16							NA							NA						
SNP7	**GG**	**G**	2	0,250	0,022	0,047	0,3667	0,421	60	2	0,538	-0,587	18,254 *	0,923	0,507	52	NA	NA	NA	NA	NA	NA	NA
	TG	**T**	11	0,750	0,022					24	0,462	-0,587					NA	NA	NA				
	TT		17							0							NA						
SNP8	**CC**	**C**	4	0,294	0,150	0,937	0,253	0,421	68	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	CG	**G**	12	0,706	0,150					NA	NA	NA					NA	NA	NA				
	GG		18							NA							NA						
SNP9	**CC**	**C**	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	GC	**G**	NA	NA	NA					NA	NA	NA					NA	NA	NA				
	GG		NA							NA							NA						
SNP10	**GG**	**G**	15	0,662	0,015	0,030	0,4412	0,454	68	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	TG	**T**	15	0,338	0,015					NA	NA	NA					NA	NA	NA				
	TT		4							NA							NA						
SNP11	**GG**	**G**	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	TG	**T**	NA	NA	NA					NA	NA	NA					NA	NA	NA				
	TT		NA							NA							NA						
SNP12	**CC**	**C**	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	TC	**T**	NA	NA	NA					NA	NA	NA					NA	NA	NA				
	TT		NA							NA							NA						
SNP13	**CC**	**C**	0	0,058	-0,061	0,064	0,115	0,111	52	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	TC	**T**	3	0,942	-0,061					NA	NA	NA					NA	NA	NA				
	TT		23							NA							NA						
SNP14	**AA**	**A**	0	0,000	0,000	X	0	0	44	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	GA	**G**	0	1,000	0,000					NA	NA	NA					NA	NA	NA				
	GG		22							NA							NA						
SNP15	**AA**	**A**	0	0,106	-0,119	0,393	0,2121	0,1925	66	0	0,000	0,000	X	1,000	1,000	48	0	0,000	0	X	0	0	36
	GA	**G**	7	0,894	-0,119					0	1,000	0,000					0	1	0				
	GG		26							24							18						
SNP16	**AA**	**A**	0	0,014	-0,014	0,000	0,378	0,028	72	0	0,000	0,000	X	1,000	1,000	58	0	0,000	0	X	0	0	58

	GA	G	1	0,986	-0,014					0	1,000	0,000					0	1	0				
	GG		35							29							29						
SNP17	CC	C	32	0,985	-0,154					27	1,000	0,000					25	0,980	-0,020				
	GC	G	1	0,015	-0,154	0,000	0,0303	0,0303	66	0	0,000	0,000	X	1,000	1,000	54	1	0,0192	-0,020	0	0,0385	0,0385	52
	GG		0							0							0						
			POP 4: Timahdite							**POP 5: Sardi**							**POP 6: Suffolk**						
	GENOTYPE	**ALLELE**	Genotype Freq	Allele Freq	Fis	x^2	H obs	H exp	n	Genotype Freq	Allele Freq	Fis	x^2	H obs	H exp	n	Genotype Freq	Allele Freq	Fis	x^2	H obs	H exp	n
SNP1	CC	C	3	0,553	-0,597					11	0,829	-0,120					9	0,577	0,055				
	TC	T	15	0,448	-0,597	6,177*	0,789	0,501	38	3	0,107	-0,120	0,130	0,214	0,198	28	12	0,423	0,055	0,143	0,462	0,498	52
	TT		1							0							5						
SNP2	CC	C	NA	NA	NA					NA	NA	NA					6	0,539	-0,238				
	GC	G	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	26	0,461	-0,238	1,242	0,615	0,507	52
	GG		NA							NA							4						
SNP3	AA	A	0	1	0					0	0,500	-1,000					0	0,981	-0,020				
	CA	C	0	0	0	x	0	0	44	22	0,500	-1,000	21,00*	1,000	0,512	44	1	0,019	-0,020	0,000	0,039	0,039	52
	CC	C	22							0							25						
SNP4	AA	A	NA	NA	NA					NA	NA	NA					6	0,019	-0,325				
	GA	G	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	17	0,981	-0,325	2,433	0,654	0,503	52
	GG		NA							NA							3						
SNP5	GG	G	0	0	0					NA	NA	NA					0	0,192	-0,020				
	TG	T	0	1	0	x	0	0	48	NA	NA	NA	NA	NA	NA	NA	1	0,981	-0,020	0,000	0,039	0,039	52
	TT		24							NA							25						
SNP6	AA	A	NA	NA	NA					0	0,000	0,000					0	0,039	-0,040				
	GA	G	NA	NA	NA	NA	NA	NA	NA	0	1,000		x	0,000	1,000	54	2	0,962	-0,040	0,020	0,769	0,075	52
	GG		NA							27							24						
SNP7	GG	G	0	0,479	-0,92					0	0,480	-0,923					8	0,460	0,436				
	TG	T	23	0,521	-0,92	19,397*	0,958	0,5098	48	24	0,520	-0,923	20,382*	0,960	0,509	50	7	0,540	0,436	5,227*	0,280	0,503	50
	TT		1							1							10						
SNP8	CC	C	NA	NA	NA					NA	NA	NA					3	0,327	0,039				
	CG	G	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	11	0,673	0,039	0,089	0,423	0,039	52

	GG		NA							NA							12						
SNP9	CC	C	NA	NA	NA					NA	NA	NA					26	1,000	0,000				
	GC	G	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	0,000		0,000	0,000	0,000	52
	GG		NA							NA							0						
SNP10	GG	G	NA	NA	NA					NA	NA	NA					14	0,750	-0,128				
	TG	T	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	11	0,250	-0,128	0,315	0,423	0,382	52
	TT		NA							NA							1						
SNP11	GG	G	NA	NA	NA					NA	NA	NA					3	0,308	0,097				
	TG	T	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	10	0,692	0,097	0,360	0,385	0,434	52
	TT		NA							NA							13						
SNP12	CC	C	NA	NA	NA					NA	NA	NA					1	0,154	0,114				
	TC	T	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	6	0,847	0,114	0,449	0,231	0,266	52
	TT		NA							NA							19						
SNP13	CC	C	NA	NA	NA					NA	NA	NA					0	0,019	-0,020				
	TC	T	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	1	0,981	-0,020	0,000	0,039	0,039	52
	TT		NA							NA							25						
SNP14	AA	A	NA	NA	NA					NA	NA	NA					8	0,580	-0,067				
	GA	G	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	13	0,420	-0,067	0,055	0,520	0,497	50
	GG		NA							NA							4						
SNP15	AA	A	0	0	0					0	0,000	0,000					0	0,000	0,000				
	GA	G	0	1	0	x	0	0	48	0	1,000		x	0,000	0,000	56	0	1,000	0,000	x	0,000	0,000	52
	GG		24							28							26						
SNP16	AA	A	0	0	0					0	0,019	-0,180					0	0,000	0,000				
	GA	G	0	1	0	x	0	0	56	1	0,982	-0,180	0,000	0,037	0,037	54	0	2,000	0,000	x	0,000	0,000	53
	GG		28							26							26						
SNP17	CC	C	12	0,816	-0,226					21	0,978	-0,223					13	0,692	0,097				
	GC	G	7	0,184	-0,226	0,813	0,368	0,309	38	1	0,923	-0,223	0,000	0,046	0,046	47	10	0,308	0,972	0,360	0,385	0,434	54
	GG		0							0							3						
		POP 7: Tetra								POP 8: Il de France							POP 9: Hungarian Tsigai						
	GENO TYPE	ALLEL E	Genotype Freq	Allele Freq	Fis	x^2	H obs	H exp	n	Genotype Freq	Allele Freq	Fis	x^2	H obs	H exp	n	Genotype Freq	Allele Freq	Fis	x^2	H obs	H exp	n

SNP1	**CC**	**C**	4	0,361	-0,084	0,175	0,500	0,468	72	4	0,429	0,028	0,057	0,476	0,502	42	8	0,561	-0,292	2,522	0,635	0,500	66
	TC	**T**	18	0,639	-0,084					10	0,571	0,028					21	0,439	-0,292				
	TT		14							7							4						
SNP2	**CC**	**C**	17	0,681	0,042	0,113	0,417	0,441	72	14	0,786	0,293	2,226	0,238	0,345	42	11	0,567	0,185	1,232	0,400	0,499	60
	GC	**G**	15	0,319	0,042					5	0,214	0,293					12	0,433	0,185				
	GG		4							2							7						
SNP3	**AA**	**A**	0	0,931	0,075	0,158	0,139	0,131	72	0	0,976	-0,024	0,000	0,048	0,048	42	0	0,906	-0,103	0,281	0,188	0,173	64
	CA	**C**	5	0,069	0,075					1	0,024	-0,024					6	0,938	-0,103				
	CC		51							20							26						
SNP4	**AA**	**A**	18	0,735	-0,058	0,064	0,412	0,395	68	15	0,810	0,382	3,712	0,191	0,316	42	11	0,578	0,039	0,097	0,469	0,496	64
	GA	**G**	14	0,265	-0,058					4	0,191	0,382					15	0,422	0,039				
	GG		2							2							6						
SNP5	**GG**	**G**	0	0,069	-0,075	0,158	0,139	0,131	72	0	0,024	-0,024	0,000	0,048	0,048	42	0	0,106	-0,119	0,393	0,212	0,193	66
	TG	**T**	5	0,931	-0,075					1	0,976	-0,024					7	0,894	-0,119				
	TT		31							20							26						
SNP6	**AA**	**A**	1	0,300	-0,293	2,737	0,543	0,426	70	3	0,452	-0,025	1,065	0,619	0,508	42	0	0,036	-0,037	0,019	0,714	0,070	56
	GA	**G**	19	0,700	-0,293					13	0,548	-0,025					2	0,964	-0,037				
	GG		15							5							26						
SNP7	**GG**	**G**	8	0,514	-0,167	0,848	0,583	0,507	72	4	0,333	0,357	3,098	0,286	0,455	42	9	0,454	0,027	2,620	0,364	0,504	66
	TG	**T**	21	0,486	-0,167					6	0,667	0,357					12	0,964	0,027				
	TT		7							11							12						
SNP8	**CC**	**C**	1	0,235	-0,155	0,732	0,417	0,366	72	1	0,214	0,010	0,026	0,333	0,345	42	6	0,313	0,418	6,095*	0,250	0,437	64
	CG	**G**	15	0,764	-0,155					7	0,786	0,010					8	0,688	0,418				
	GG		20							13							18						
SNP9	**CC**	**C**	36	1,000	0,000	x	0,000	0,000	72	20	0,952	1,000	41,026	0,000	0,093	42	30	0,909	1,000	39,051	0,000	0,168	66
	GC	**G**	0	0,000	0,000					0	0,048	1,000					0	0,091	1,000				
	GG		0							1							3						
SNP10	**GG**	**G**	21	0,729	0,350	4,721*	0,257	0,401	70	7	0,595	0,213	0,083	0,524	0,494	42	18	0,719	0,227	1,919	0,313	0,411	64
	TG	**T**	9	0,271	0,350					11	0,405	0,213					10	0,281	0,227				
	TT		5							3							4						
SNP11	**GG**	**G**	0	0,056	-0,059	0,092	0,111	0,106	72	2	0,238	-0,129	1,227	0,286	0,372	40	0	0,076	-0,082	0,175	0,152	0,142	66

	TG	T	4	0,944	-0,059					6	0,762	-0,129					5	-0,924	-0,082				
	TT		32							13							28						
SNP12	CC	C	1	0,208	-0,095					0	0,071	-0,077					9	0,550	-0,010				
	TC	T	13	0,792	-0,095	0,241	0,361	0,335	72	3	0,691	-0,077	0,081	0,143	0,136	42	15	0,450	-0,010	0,001	0,500	0,503	60
	TT		22							18							6						
SNP13	CC	C	0	0,000	0,000					1	0,310	-0,226					0	0,032	-0,033				
	TC	T	0	1,000	0,000	0,000	0,000	0,000	72	11	0,691	-0,226	0,865	0,524	0,438	42	2	0,968	-0,033	0,017	0,065	0,064	62
	TT		36							9							29						
SNP14	AA	A	1	0,439	0,077					1	0,310	-0,226					4	0,364	-0,048				
	GA	G	11	0,561	0,077	0,284	0,455	0,500	66	11	0,691	-0,226	0,865	0,524	0,438	42	16	0,636	-0,048	0,034	0,485	0,470	66
	GG		9							9							13						
SNP15	AA	A	0	0,028	-0,029					0	0,000	0,000					0	0,167	-0,200				
	GA	G	2	0,972	-0,029	0,014	0,056	0,055	72	0	1,000	0,000	x	0,000	0,000	42	11	0,833	-0,200	1,185	0,333	0,282	66
	GG		34							21							22						
SNP16	AA	A	0	0,083	-0,091					0	0,050	-0,053					0	0,000	0,000				
	GA	G	2	0,917	-0,091	0,244	0,167	0,155	72	2	0,950	-0,053	0,027	0,100	0,097	40	0	1,000	0,000	x	0,000	0,000	66
	GG		18							18							33						
SNP17	CC	C	16	0,958	-0,044					16	0,881	-0,135					10	0,546	0,022				
	GC	G	5	0,042	-0,044	0,045	0,083	0,081	72	5	0,119	-0,135	0,300	0,238	0,215	42	16	0,455	0,022	0,047	0,485	0,504	66
	GG		0							0							7						

	POP 10: Hungarian Racka									POP 11: Karakul							POP 12: Romanian Racka						
	GENOTYPE	ALLELE	Genotype Freq	Allele Freq	Fis	x^2	H obs	H exp	n	Genotype Freq	Allele Freq	Fis	x^2	H obs	H exp	n	Genotype Freq	Allele Freq	Fis	x^2	H obs	H exp	n
SNP1	CC	C	8	0,333	0,250					2	0,265	0,244					14	0,525	-0,183				
	TC	T	16	0,667	0,250	3,276	0,333	0,449	96	5	0,735	0,244	1,333	0,294	0,401	34	36	0,475	-0,183	1,867	0,590	0,503	112
	TT		24							10							11						
SNP2	CC	C	16	0,610	0,078					3	0,667	0,250					39	0,814	-0,006				
	GC	G	18	-0,390	0,078	0,332	0,439	0,482	82	2	0,333	0,250	0,744	0,333	0,485	12	18	0,186	-0,006	0,000	0,305	0,306	118
	GG		7							1							2						
SNP3	AA	A	0	0,969	-0,032					0	0,917	-0,091					2	0,879	0,166				
	CA	C	3	0,031	-0,032	0,033	0,063	0,061	96	3	0,083	-0,091	0,097	0,167	0,157	36	11	0,121	0,166	1,962	0,177	0,214	124

	CC		45							15							49					
SNP4	**AA**	**A**	24	0,734	-0,144	0,849	0,447	0,395	94	5	0,636	0,214	0,769	0,364	0,485	22	42	0,836	-0,077	0,292	0,295	0,276	122
	GA	**G**	21	0,266	-0,144					4	0,364	0,214					18	0,164	-0,077				
	GG		2							2							1						
SNP5	**GG**	**G**	0	0,031	-0,032	0,032	0,063	0,061	96	0	0,115	-0,130	0,142	0,231	0,212	26	2	0,121	0,166	1,962	0,177	0,214	124
	TG	**T**	3	0,969	-0,032					3	0,884	-0,130					11	0,879	0,166				
	TT		45							10							49						
SNP6	**AA**	**A**	12	0,476	0,236	2,592	0,381	0,505	94	0	0,000	0,000	0,000	0,000	0,000	12	16	0,451	0,239	3,717	0,377	0,499	122
	GA	**G**	16	0,524	0,236					0	1,000	0,000					23	0,549	0,239				
	GG		14							6							22						
SNP7	**GG**	**G**	26	0,719	0,124	0,878	0,354	0,409	96	7	0,531	0,624	6,892 *	0,119	0,514	32	7	0,367	-0,077	0,279	0,500	0,468	120
	TG	**T**	17	0,281	0,124					3	0,469	0,624					30	0,633	-0,077				
	TT		5							6							23						
SNP8	**CC**	**C**	2	0,229	1,000	0,127	0,375	0,357	96	2	0,167	0,760	10,752*	0,067	0,287	30	5	0,328	-0,116	0,709	0,492	0,444	122
	CG	**G**	18	0,771	1,000					1	0,833	0,760					30	0,672	-0,116				
	GG		28							12							26						
SNP9	**CC**	**C**	39	0,830	1,000	49,7038*	0,000	0,286	94	14	0,933	1,000	29,037*	0,000	0,287	31	61	1,000	0,000	x	0,000	0,000	122
	GC	**G**	0	0,170	1,000					0	0,067	1,000					0	0,000	0,000				
	GG		8							1							0						
SNP10	**GG**	**G**	18	0,646	-0,184	1,451	0,542	0,462	96	11	0,833	0,280	1,685	0,200	0,287	32	14	0,524	-0,196	2,196	0,597	0,503	124
	TG	**T**	26	0,354	-0,184					3	0,167	0,280					37	0,476	-0,196				
	TT		4							1							11						
SNP11	**GG**	**G**	0	0,073	-0,079	0,252	0,146	0,137	96	2	0,083	1,000	31,380*	0,000	0,156	48	0	0,117	-0,132	0,964	0,233	0,208	120
	TG	**T**	7	0,927	-0,079					0	0,917	1,000					14	0,883	-0,132				
	TT		41							22							46						
SNP12	**CC**	**C**	4	0,351	-0,167	1,159	0,532	0,461	94	0	0,091	-0,100	0,053	0,182	0,173	22	12	0,444	-0,013	0,001	0,500	0,498	124
	TC	**T**	25	0,650	-0,167					2	0,909	-0,100					31	0,557	-0,013				
	TT		18							9							19						
SNP13	**CC**	**C**	0	0,000	0,000	x	0,000	0,000	96	0	0,083	-0,100	0,000	0,167	0,167	12	0	0,008	-0,008	0,000	0,016	0,016	122
	TC	**T**	0	1,000	0,000					1	0,917	-0,100					1	0,992	-0,008				
	TT		48							5							60						

SNP14	AA	A	3	0,240	0,028					2	0,409	-0,091					18	0,508	0,129				
	GA	G	17	0,760	0,028	0,071	0,354	0,368	96	5	0,591	-0,091	0,128	0,455	0,507	22	27	0,492	0,129	1,162	0,436	0,504	124
	GG		28							4							17						
SNP15	AA	A	0	0,208	-0,263					0	0,000	0,060					0	0,057	-0,060				
	GA	G	20	0,760	-0,263	3,133	0,417	0,333	96	0	1,000	0,060	x	0,000	0,000	24	7	0,944	-0,060	0,189	0,113	0,107	124
	GG		28							12							55						
SNP16	AA	A	0	0,000						2	0,250	0,429					0	0,032	-0,033				
	GA	G	0	1,000		x	0,000	0,000	96	3	0,750	0,429	3,210	0,214	0,389	28	4	0,968	-0,033	0,051	0,065	0,063	124
	GG		48							9							58						
SNP17	CC	C	33	0,843	0,185					13	1,000	0,000					49	0,887	0,034				
	GC	G	15	0,156	0,185	1,523	0,313	0,266	96	0	0,000	0,000	x	0,000	0,000	26	12	0,113	0,034	0,114	0,194	0,202	124
	GG		0							0							1						
	POP 13: Romanian Merino									**POP 14: Romanian Tsigai**							**POP 15: Turcana**						
	GENOTYPE	**ALLELE**	Genotype Freq	Allele Freq	Fis	x^2	H obs	H exp	n	Genotype Freq	Allele Freq	Fis	x^2	H obs	H exp	n	Genotype Freq	Allele Freq	Fis	x^2	H obs	H exp	n
SNP1	CC	C	20	0,655	0,210					1	0,367	-0,292					8	0,412	-0,122				
	TC	T	15	0,345	0,210	2,083	0,357	0,458	84	9	0,633	-0,292	1,006	0,600	0,461	30	31	0,5877	-0,122	0,734	0,544	0,489	114
	TT		7							5							18						
SNP2	CC	C	11	0,571	-0,050					5	0,682	-0,048					32	0,763	-0,116				
	GC	G	8	0,429	-0,050	0,044	0,514	0,497	70	5	0,318	-0,048	0,000	0,455	0,455	22	23	0,2368	-0,116	0,666	0,404	0,365	11
	GG		6							1							2						
SNP3	AA	A	9	0,977	-0,023					0	1,000	0,000					0	0,9386	-0,654				
	CA	C	2	0,0227	-0,023	0,012	0,046	0,050	88	0	0,000	0,000	x	0,000	0,000	34	7	0,614	-0,654	0,207	0,123	0,116	114
	CC		42							17							50						
SNP4	AA	A	12	0,566	-0,018					7	0,692	0,278					37	0,810	-0,122				
	GA	G	19	0,4342	-0,018	0,0007	0,500	0,498	76	4	0,308	0,278	1,355	0,308	0,443	26	20	0,1897	-0,122	0,759	0,345	0,310	116
	GG		7							2							1						
SNP5	GG	G	0	0,012	-0,012					0	0,000	0,000					0	0,060	-0,064				
	TG	T	1	0,9881	-0,012	0,000	0,024	0,024	84	0	1,000	0,000	x	0,000	0,000	32	7	0,9397	-0,064	0,203	0,121	0,114	116
	TT		41							16							51						
SNP6	AA	A	0	0,074	-0,079	0,169	0,147	0,138	68	3	0,318	0,791		0,091	0,455	22	2	0,164	0,056	0,249	0,259	0,276	116

	GA	**G**	5	0,9265	-0,079					1	0,682	0,791	8,000 *				15	0,1832	0,056				
	GG		29							7							41						
SNP7	**GG**	**G**	5	0,361	-0,059					2	0,158	0,042					7	0,272	0,247				
	TG	**T**	21	0,6395	-0,059	0,097	0,488	0,467	86	2	0,842	0,042	8,422 *	0,105	0,273	38	17	0,728	0,247	3,761	0,298	0,400	114
	TT		17							15							33						
SNP8	**CC**	**C**	2	0,140	0,225					3	0,375	0,200					9	0,371	0,076				
	CG	**G**	8	0,8605	0,225	2,548	0,186	0,243	86	6	0,625	0,200	0,872	0,375	0,484	40	25	0,629	0,076	0,419	0,431	0,471	116
	GG		33							7							24						
SNP9	**CC**	**C**	39	0,975	1,000					20	1,000	0,000					53	0,930	1,000				
	GC	**G**	0	0,025	1,000	79,013*	0,000	0,049	80	0	0,000	0,000	x	0,000	0,000	40	0	0,070	1,000	64,610*	0,000	0,132	114
	GG		1							0							4						
SNP10	**GG**	**G**	30	0,807	0,198					11	0,775	-0,290					26	0,655	0,084				
	TG	**T**	11	0,1932	0,198	1,988	0,250	0,515	88	9	0,225	-0,290	1,471	0,450	0,358	40	24	0,345	0,084	0,503	0,414	0,456	116
	TT		3							7							8						
SNP11	**GG**	**G**	4	0,275	0,122					0	0,056	-0,059					2	0,098	0,294				
	TG	**T**	14	0,725	0,122	0,728	0,350	0,404	80	2	0,944	-0,059	0,030	0,111	0,108	36	7	0,902	0,294	5,523*	0,125	0,179	112
	TT		22							16							47						
SNP12	**CC**	**C**	1	0,060	0,362					0	0,125	-0,143					1	0,147	-0,034				
	TC	**T**	3	0,9405	0,362	7,092 *	0,071	0,113	84	4	0,875	-0,143	0,238	0,250	0,226	32	15	0,853	-0,034	0,0381	0,259	0,252	116
	TT		38							12							42						
SNP13	**CC**	**C**	1	0,071	0,282					0	0,000	0,000					1	0,089	0,122				
	TC	**T**	4	0,9286	0,282	4,208 *	0,095	0,134	84	0	1,000	0,000	x	0,000	0,000	26	8	0,911	0,122	1,034	0,143	0,164	112
	TT		37							13							47						
SNP14	**AA**	**A**	12	0,544	-0,120					5	0,633	-0,292					11	0,431	0,016				
	GA	**G**	25	0,4556	-0,120	0,532	0,556	0,502	90	9	0,367	-0,292	1,006	0,600	0,480	30	28	0,569	0,016	0,035	0,483	0,495	116
	GG		8							1							19						
SNP15	**AA**	**A**	0	0,022	0,227					0	0,000	0,000					0	0,139	-0,161				
	GA	**G**	2	0,9778	0,227	0,011	0,044	0,044	90	0	1,000	0,000	x	0,000	0,000	32	15	0,861	-0,161	1,301	0,278	0,241	108
	GG		43							16							39						
SNP16	**AA**	**A**	0	0,000	0					0	0,028	-0,286					0	0,009	-0,009				
	GA	**G**	0	1	0	x	0	0	90	1	0,972	-0,286	0,000	0,056	0,056	36	1	0,861	-0,009	0,000	0,017	0,017	116

	GG		45							17							57						
SNP17	CC	C	29	0,8295	-0,206	1,718	0,341	0,286	88	12	0,853	-0,172	0,394	0,294	0,259	34	45	0,884	0,217	3,014	0,161	0,207	112
	GC	G	15	0,1705	-0,206					5	0,147	-0,172					9	0,116	0,217				
	GG		0							0							2						

			POP 16: Hungarian Merino							POP 17: Hungarian Awassi						
	GENOTYPE	ALLELE	Genotype Freq	Allele Freq	Fis	x^2	H obs	H exp	n	Genotype Freq	Allele Freq	Fis	x^2	H obs	H exp	n
SNP1	CC	C	16	0,686	-0,061	0,075	0,457	0,437	70	4	0,338	-0,062	0,099	0,475	0,453	80
	TC	T	16	0,314	-0,061					19	0,662					
	TT		3							17						
SNP2	CC	C	11	0,557	0,016	0,032	0,486	0,501	70	30	0,875	-0,143	0,727	0,250	0,222	80
	GC	G	17	0,443	0,016					10	0,125					
	GG		7							0						
SNP3	AA	A	0	0,000	0,000	x	0,000	0,000	70	3	0,175	0,307	4,259 *	0,200	0,292	80
	CA	C	0	1	0,000					8	0,825					
	CC		35							29						
SNP4	AA	A	11	0,588	-0,093	0,6490	0,529	0,492	68	30	0,885	-0,130	0,583	0,231	0,207	78
	GA	G	18	0,412	-0,093					9	0,115					
	GG		5							0						
SNP5	GG	G	0	0,000	0,000	x	0,000	0,000	70	3	0,175	0,307	4,259 *	0,200	0,292	80
	TG	T	0	1	0,000					8	0,825					
	TT		35							29						
SNP6	AA	A	2	0,143	0,300	3,692	0,171	0m248	70	0	0,063	-0,067	0,141	0,125	0,119	80
	GA	G	6	0,857	0,300					5	0,937					
	GG		27							35						
SNP7	GG	G	2	0,243	-0,010	0,001	0,371	0,373	70	5	0,313	0,127	0,790	0,375	0,435	80
	TG	T	13	0,757	-0,010					15	0,687					
	TT		20							20						
SNP8	CC	C	1	0,171	-0,006	0,003	0,286	0,288	70	3	0,337	-0,174	1,048	0,525	0,453	80
	CG	G	10	0,829	-0,006					21	0,663					
	GG		24							16						

Notes:

NA = not available data;

* = deviated from HWE;

SNP1= rs161504783-*HSPA12A*;

SNP2= rs397514116-*HSP90AA1*;

SNP3= rs397514117-*HSP90AA1*;

SNP4= rs397514269 -*HSP90AA1*;

SNP5= rs397514272 -*HSP90AA1*;

SNP6= rs397514273-*HSP90AA1*;

SNP7= rs410259751-*IL33*;

SNP8= rs411181557-*DIO2*;

SNP9= rs414917134-*BTNL2*;

SNP10= rs416941267-*CSN2*;

SNP11= rs420611298-*ABCG1*;

SNP12= rs420959261-*CSN1S1*;

SNP13= rs430298704-*CSN2*;

SNP14= rs55631463-*GHR*;

SNP15= rs588145625-*HSPA8*;

SNP16= rs588498137-*STAT3*;

SNP17= rs602521720-*HCRT*

SNP9	**CC**	**C**	18	0,765	-0,301	2,986	0,471	0,365	68	24	0,811	-0,057	0,072	0,324	0,311	74
	GC	**G**	16	0,235	-0,301					12	0,189					
	GG		0							1						
SNP10	**GG**	**G**	26	0,857	0,067	0,246	0,229	0,248	70	26	0,813	-0,067	0,121	0,325	0,309	80
	TG	**T**	8	0,143	0,067					13	0,187					
	TT		1							1						
SNP11	**GG**	**G**	0	0,000	0,000	x	0,000	0,000	68	0	0,000	0,000	x	0,000	0,000	80
	TG	**T**	0	1	0,000					0	1,000					
	TT		35							40						
SNP12	**CC**	**C**	1	0,129	0,108	0,562	0,200	0,227	70	1	0,154	0,015	0,032	0,256	0,264	78
	TC	**T**	7	0,871	0,108					10	0,846					
	TT		27							28						
SNP13	**CC**	**C**	0	0,143	-0,167	0,864	0,286	0,248	79	0	0,116	-0,130	0,583	0,231	0,207	78
	TC	**T**	10	0,857	-0,167					9	0,885					
	TT		25							30						
SNP14	**AA**	**A**	0	0,264	-0,360	4,122 *	0,529	0,395	68	11	0,563	-0,168	0,969	0,575	0,498	80
	GA	**G**	18	0,735	-0,360					23	0,437					
	GG		16							6						
SNP15	**AA**	**A**	0	0,000	0,000	x	0,000	0,000	70	0	0,000	0,000	x	0,000	0,000	80
	GA	**G**	0	1	0,000					0	1,000					
	GG		35							40						
SNP16	**AA**	**A**	0	0,157	-0,186	1,093	0,314	0,269	70	0	0,013	-0,013	0,000	0,025	0,025	80
	GA	**G**	11	0,843	-0,186					1	0,987					
	GG		24							39						
SNP17	**CC**	**C**	35	1	0,000	x	0,000	0,000	68	37	0,963	-0,039	0,040	0,075	0,073	80
	GC	**G**	0	0	0,000					3	0,037					
	GG		0							0						

Appendix Table S3. PCA loading values

Dim.1	Dim.2	Dim.3	Dim.4	Dim.5
a	-0.0957268773	-0.22803507	-0.220966246	0.17833003	0.30942823
b	-0.1098545778	0.21088758	0.190115964	0.01645028	-0.49373068
c	0.9018327643	0.28972654	0.006134796	0.04065301	0.04521233
d	-0.3097836631	0.32183409	0.636760466	-0.22322588	0.09160223
e	0.8583257228	0.29329530	0.072939161	0.04691957	0.03959363
f	-0.0433820120	0.54614718	0.485507841	-0.03031388	0.16065120
g	0.0809617873	0.15106806	-0.228951900	0.37115209	0.23628456
h	0.0008438631	-0.17435902	-0.113039462	0.06780223	0.02349125
i	-0.0808817477	0.29922171	-0.267107244	-0.38865221	0.35386320
j	0.2983367091	-0.53908887	0.148228003	-0.34371408	-0.02507619
k	-0.0190776933	-0.03781087	0.338941220	0.48522345	0.16514847
l	0.2875181213	-0.42048722	0.235140415	-0.40438354	0.12178878
m	-0.1179450424	0.32688286	-0.165418159	0.16382153	0.37643513
n	-0.1235961207	-0.15956549	0.262046228	-0.06285451	0.53594740
o	-0.1283062908	0.42438807	-0.220599976	-0.11924405	-0.36220706
p	0.0393344866	-0.27721318	0.242586815	0.51990040	-0.10647321
q	0.0670516758	-0.16383538	0.247734410	0.20590467	-0.15792399

Appendix Table S4. PCA score values

ID	Breed	Characteristic	PCA1	PCA2
PRA 1	Pramenka	Cold	-0,415607925	2,005842275
PRA 2	Pramenka	Cold	-0,228404635	0,13477435
PRA 3	Pramenka	Cold	-0,018777046	-1,008203937
PRA 4	Pramenka	Cold	0,028482393	-0,037063831
PRA 5	Pramenka	Cold	-0,430075936	1,334005038
PRA 6	Pramenka	Cold	-0,073255595	-2,109754132
PRA 7	Pramenka	Cold	-0,491988925	-0,127640756
PRA 8	Pramenka	Cold	-0,032256603	-1,043445607
PRA 9	Pramenka	Cold	0,507646951	-1,38845705
PRA 10	Pramenka	Cold	-0,354420922	0,129341152
PRA 11	Pramenka	Cold	1,595279355	0,396832115
PRA 12	Pramenka	Cold	-1,213576232	1,367590945
PRA 13	Pramenka	Cold	-0,233806037	-0,824072246
PRA 14	Pramenka	Cold	-0,278864954	-0,083346219
PRA 15	Pramenka	Cold	-0,601692592	0,080856033
PRA 16	Pramenka	Cold	-0,226718705	-1,198943552
PRA 17	Pramenka	Cold	-0,619191851	-0,515526486
PRA 18	Pramenka	Cold	-0,821246135	0,947141067
PRA 19	Pramenka	Cold	-0,548134682	-1,407469958
PRA 20	Pramenka	Cold	-0,064086413	0,01332179
PRA 21	Pramenka	Cold	0,243888132	-2,137218047
PRA 22	Pramenka	Cold	-0,579718943	0,55660855
PRA 23	Pramenka	Cold	0,41370938	-0,465407141
PRA 24	Pramenka	Cold	1,725294481	0,193018388
PRA 25	Pramenka	Cold	-0,169279349	0,427327971
PRA 26	Pramenka	Cold	-0,382104038	-0,764818766
PRA 27	Pramenka	Cold	-0,215381631	-0,052944425
PRA 28	Pramenka	Cold	-1,034244587	0,485630093

PRA 29	Pramenka	Cold	0,15113485	-2,594946563
PRA 30	Pramenka	Cold	-0,252662316	-0,54811174
PRA 31	Pramenka	Cold	-0,762363927	0,205980361
PRA 32	Pramenka	Cold	-0,350495321	-0,559932055
PRA 33	Pramenka	Cold	-0,574771374	0,522227845
PRA 34	Pramenka	Cold	1,494132034	-0,193991868
PRA 35	Pramenka	Cold	-0,696707751	0,956165286
PRA 36	Pramenka	Cold	-0,101747216	-1,225831541
PRA 37	Pramenka	Cold	-0,52178537	-0,118616673
BG 1	Beni Guil	Hot	-0,028539759	-0,068678003
BG 2	Beni Guil	Hot	-0,063456557	0,05375226
BG 3	Beni Guil	Hot	-0,063456557	0,05375226
BG 4	Beni Guil	Hot	-0,044356357	0,004278476
BG 5	Beni Guil	Hot	-0,063456557	0,05375226
BG 6	Beni Guil	Hot	-0,063456557	0,05375226
BG 7	Beni Guil	Hot	-0,063456557	0,05375226
BG 8	Beni Guil	Hot	-0,109925377	-0,038164218
BG 9	Beni Guil	Hot	0,034259665	0,089907166
BG 10	Beni Guil	Hot	-0,000657133	0,212337429
BG 11	Beni Guil	Hot	-0,063456557	0,05375226
BG 12	Beni Guil	Hot	-0,063456557	0,05375226
BG 13	Beni Guil	Hot	-0,044356357	0,004278476
BG 14	Beni Guil	Hot	-0,063456557	0,05375226
BG 15	Beni Guil	Hot	-0,063456557	0,05375226
BG 16	Beni Guil	Hot	-0,063456557	0,05375226
BG 17	Beni Guil	Hot	-0,047125953	0,120420952
BG 18	Beni Guil	Hot	-0,237674156	-0,290854465
BG 19	Beni Guil	Hot	-0,028539759	-0,068678003
BG 20	Beni Guil	Hot	-0,019879642	0,189225099
BG 21	Beni Guil	Hot	-0,063456557	0,05375226
BG 22	Beni Guil	Hot	-0,028539759	-0,068678003
BG 23	Beni Guil	Hot	-0,063456557	0,05375226
BG 24	Beni Guil	Hot	0,116634913	0,508530894
BG 25	Beni Guil	Hot	-0,000657133	0,212337429
BG 26	Beni Guil	Hot	-0,063456557	0,05375226
BG 27	Beni Guil	Hot	-0,028539759	-0,068678003
BG 28	Beni Guil	Hot	-0,237674156	-0,290854465
BG 29	Beni Guil	Hot	-0,000657133	0,212337429
DM 1	D'man	Hot	-0,109925377	-0,038164218
DM 2	D'man	Hot	-0,047125953	0,120420952
DM 3	D'man	Hot	-0,109925377	-0,038164218
DM 4	D'man	Hot	-0,012209155	-0,002009312
DM 5	D'man	Hot	-0,075008579	-0,160594481
DM 6	D'man	Hot	-0,075008579	-0,160594481
DM 7	D'man	Hot	-0,075008579	-0,160594481
DM 8	D'man	Hot	-0,075008579	-0,160594481
DM 9	D'man	Hot	-0,047125953	0,120420952
DM 10	D'man	Hot	-0,028025753	0,070947167
DM 11	D'man	Hot	-0,109925377	-0,038164218
DM 12	D'man	Hot	-0,075008579	-0,160594481
DM 13	D'man	Hot	-0,075008579	-0,160594481
DM 14	D'man	Hot	-0,075008579	-0,160594481
DM 15	D'man	Hot	-0,075008579	-0,160594481
DM 16	D'man	Hot	-0,109925377	-0,038164218
DM 17	D'man	Hot	-0,028025753	0,070947167
DM 18	D'man	Hot	-0,019879642	0,189225099
DM 19	D'man	Hot	0,006891045	-0,051483096
DM 20	D'man	Hot	-0,109925377	-0,038164218
DM 21	D'man	Hot	-0,109925377	-0,038164218
DM 22	D'man	Hot	-0,109925377	-0,038164218
DM 23	D'man	Hot	-0,109925377	-0,038164218
DM 24	D'man	Hot	-0,019879642	0,189225099
DM 25	D'man	Hot	0,071083065	-0,185766803
DM 26	D'man	Hot	-0,019879642	0,189225099

DM 27	D'man	Hot	-0,075008579	-0,160594481
DM 28	D'man	Hot	-0,109925377	-0,038164218
DM 29	D'man	Hot	-0,109925377	-0,038164218
TIM 1	Timahdite	Hot	0,116634913	0,508530894
TIM 2	Timahdite	Hot	-0,115277138	0,170817888
TIM 3	Timahdite	Hot	-0,405824065	-0,065330361
TIM 4	Timahdite	Hot	-0,308107843	-0,029175455
TIM 5	Timahdite	Hot	-0,452292885	-0,157246838
TIM 6	Timahdite	Hot	-0,225732595	0,389448273
TIM 7	Timahdite	Hot	-0,044356357	0,004278476
TIM 8	Timahdite	Hot	-0,059867544	-0,293955036
TIM 9	Timahdite	Hot	-0,000657133	0,212337429
TIM 10	Timahdite	Hot	-0,398275887	-0,329150886
TIM 11	Timahdite	Hot	-0,224815623	-0,212932945
TIM 12	Timahdite	Hot	-0,224815623	-0,212932945
TIM 13	Timahdite	Hot	-0,287615047	-0,371518115
TIM 14	Timahdite	Hot	-0,225732595	0,389448273
TIM 15	Timahdite	Hot	-0,386723865	-0,114804145
TIM 16	Timahdite	Hot	-0,386723865	-0,114804145
TIM 17	Timahdite	Hot	-0,287615047	-0,371518115
TIM 18	Timahdite	Hot	-0,370907267	-0,187760624
TIM 19	Timahdite	Hot	-0,343024641	0,093254809
TIM 20	Timahdite	Hot	-0,433192685	-0,206720623
TIM 21	Timahdite	Hot	-0,343024641	0,093254809
TIM 22	Timahdite	Hot	-0,386723865	-0,114804145
TIM 23	Timahdite	Hot	-0,287615047	-0,371518115
TIM 24	Timahdite	Hot	-0,370393261	-0,048135453
TIM 25	Timahdite	Hot	-0,386723865	-0,114804145
TIM 26	Timahdite	Hot	-0,405824065	-0,065330361
TIM 27	Timahdite	Hot	0,018443068	0,162863645
TIM 28	Timahdite	Hot	-0,206206913	-0,352710743
SAR 1	Sardi	Hot	0,243373253	0,574269171
SAR 2	Sardi	Hot	0,224273053	0,623742955
SAR 3	Sardi	Hot	0,224273053	0,623742955
SAR 4	Sardi	Hot	0,224273053	0,623742955
SAR 5	Sardi	Hot	0,341565099	0,91993642
SAR 6	Sardi	Hot	0,341565099	0,91993642
SAR 7	Sardi	Hot	0,341565099	0,91993642
SAR 8	Sardi	Hot	0,278290051	0,451838908
SAR 9	Sardi	Hot	0,224273053	0,623742955
SAR 10	Sardi	Hot	0,341565099	0,91993642
SAR 11	Sardi	Hot	0,341565099	0,91993642
SAR 12	Sardi	Hot	0,224273053	0,623742955
SAR 13	Sardi	Hot	0,341565099	0,91993642
SAR 14	Sardi	Hot	0,224273053	0,623742955
SAR 15	Sardi	Hot	-0,012910719	0,540817491
SAR 16	Sardi	Hot	-0,025119874	0,538808179
SAR 17	Sardi	Hot	0,045703352	0,743085165
SAR 18	Sardi	Hot	0,334674053	0,971419516
SAR 19	Sardi	Hot	0,177804232	0,531826478
SAR 20	Sardi	Hot	-0,025119874	0,538808179
SAR 21	Sardi	Hot	-0,006019673	0,489334394
SAR 22	Sardi	Hot	0,341565099	0,91993642
SAR 23	Sardi	Hot	-0,006019673	0,489334394
SAR 24	Sardi	Hot	-0,087919298	0,38022301
SAR 25	Sardi	Hot	0,202266973	0,205355728
SAR 26	Sardi	Hot	0,341565099	0,91993642
SAR 27	Sardi	Hot	0,459774117	0,613748665
SAR 28	Sardi	Hot	0,019122995	1,182799756
SAR 29	Sardi	Hot	0,16148217	0,003061571
SUF 1	Suffolk	Cold	-0,247754652	-0,915656329
SUF 2	Suffolk	Cold	-0,353324314	0,593889988
SUF 3	Suffolk	Cold	-0,195400822	0,270717103
SUF 4	Suffolk	Cold	-0,343858655	0,796856486

SUF 5	Suffolk	Cold	-0,767242474	0,885162373
SUF 6	Suffolk	Cold	-0,956443603	0,599680073
SUF 7	Suffolk	Cold	-0,987791088	1,719315732
SUF 8	Suffolk	Cold	-0,060723385	-0,23720826
SUF 9	Suffolk	Cold	-0,551473667	0,245795614
SUF 10	Suffolk	Cold	-0,774428241	0,140423479
SUF 11	Suffolk	Cold	-0,796579079	1,377200401
SUF 12	Suffolk	Cold	-0,058169553	0,415354994
SUF 13	Suffolk	Cold	-0,563393766	-0,403264887
SUF 14	Suffolk	Cold	-0,346204989	0,709649933
SUF 15	Suffolk	Cold	0,238290901	-0,861030167
SUF 16	Suffolk	Cold	-0,86958207	1,413127978
SUF 17	Suffolk	Cold	-0,724124565	-0,832408308
SUF 18	Suffolk	Cold	0,048611465	-0,748561566
SUF 19	Suffolk	Cold	-1,070954753	1,112786868
SUF 20	Suffolk	Cold	-0,800070829	2,329159094
SUF 21	Suffolk	Cold	3,568658527	2,244676972
SUF 22	Suffolk	Cold	-0,551641748	0,599496038
SUF 23	Suffolk	Cold	-0,240594815	-0,691722505
SUF 24	Suffolk	Cold	-0,854598539	1,354003553
SUF 25	Suffolk	Cold	-1,05115441	1,282710407
SUF 26	Suffolk	Cold	-0,09480667	-1,300933908
BTET 1	Bábolna Tetra	Cold	0,300557605	-1,452473773
BTET 2	Bábolna Tetra	Cold	-1,102977495	1,492611757
BTET 3	Bábolna Tetra	Cold	4,766131019	0,025550834
BTET 4	Bábolna Tetra	Cold	0,168614473	-0,342035309
BTET 5	Bábolna Tetra	Cold	0,416703694	-1,62649576
BTET 6	Bábolna Tetra	Cold	0,382308044	-1,36964942
BTET 7	Bábolna Tetra	Cold	0,415498824	-3,709225987
BTET 8	Bábolna Tetra	Cold	-0,799338551	0,729118061
BTET 9	Bábolna Tetra	Cold	-0,012690741	-1,031133909
BTET 10	Bábolna Tetra	Cold	-0,569752634	-0,429824069
BTET 11	Bábolna Tetra	Cold	-0,552400872	0,121093133
BTET 12	Bábolna Tetra	Cold	-0,407641503	-0,613978939
BTET 13	Bábolna Tetra	Cold	-0,518843492	-0,557861542
BTET 14	Bábolna Tetra	Cold	-1,15324674	1,16953147
BTET 15	Bábolna Tetra	Cold	-0,368351077	-1,094508316
BTET 16	Bábolna Tetra	Cold	0,651232083	-1,88478607
BTET 17	Bábolna Tetra	Cold	-1,034690009	0,899224919
BTET 18	Bábolna Tetra	Cold	-0,861998929	0,488380236
BTET 19	Bábolna Tetra	Cold	3,490659387	1,903077275
BTET 20	Bábolna Tetra	Cold	-0,955849895	1,367581312
BTET 21	Bábolna Tetra	Cold	-0,736925892	0,393368374
BTET 22	Bábolna Tetra	Cold	4,105450777	0,736797182
BTET 23	Bábolna Tetra	Cold	0,516151078	-1,873966006
BTET 24	Bábolna Tetra	Cold	-0,874476173	1,821537072
BTET 25	Bábolna Tetra	Cold	-1,095798083	0,098244905
BTET 26	Bábolna Tetra	Cold	-1,055861424	0,958128844
BTET 27	Bábolna Tetra	Cold	-0,234904775	0,054988849
BTET 28	Bábolna Tetra	Cold	-0,077757395	-1,206041709
BTET 29	Bábolna Tetra	Cold	3,17286869	2,352347812
BTET 30	Bábolna Tetra	Cold	-0,839868538	-0,591170593
BTET 31	Bábolna Tetra	Cold	0,084081815	-0,059407405
BTET 32	Bábolna Tetra	Cold	3,752777399	2,117670923
BTET 33	Bábolna Tetra	Cold	0,140755453	0,132744515
BTET 34	Bábolna Tetra	Cold	-0,539495783	0,949572397
BTET 35	Bábolna Tetra	Cold	-0,127044201	0,370095838
BTET 36	Bábolna Tetra	Cold	-1,100896694	1,196338225
IDF 1	Ile de France	Cold	0,17923387	-1,887826504
IDF 2	Ile de France	Cold	0,061391644	-2,300505554
IDF 3	Ile de France	Cold	0,292777816	-1,283594607
IDF 4	Ile de France	Cold	0,247767351	-1,749193305
IDF 5	Ile de France	Cold	0,015863742	-1,157232993
IDF 6	Ile de France	Cold	-0,481146334	0,296221433

IDF 7	Ile de France	Cold	-1,339077546	0,387038644
IDF 8	Ile de France	Cold	-0,02349922	-1,092647802
IDF 9	Ile de France	Cold	0,103838816	-1,333306537
IDF 10	Ile de France	Cold	-1,19680114	1,083379789
IDF 11	Ile de France	Cold	3,623657385	2,541948634
IDF 12	Ile de France	Cold	0,064815445	-0,788640955
IDF 13	Ile de France	Cold	0,220934294	-0,649972842
IDF 14	Ile de France	Cold	-0,477091998	-0,235551316
IDF 15	Ile de France	Cold	-0,563806656	0,462845212
IDF 16	Ile de France	Cold	-0,672347668	0,176709342
IDF 17	Ile de France	Cold	0,104579252	-1,495487068
IDF 18	Ile de France	Cold	-1,042695133	0,556202274
IDF 19	Ile de France	Cold	-0,490639415	0,306281369
IDF 20	Ile de France	Cold	0,000920221	-0,875068809
IDF 21	Ile de France	Cold	0,035324167	-2,017340775
HUTSI 1	Hu Tsigai	Cold	0,014100565	-0,758903932
HUTSI 2	Hu Tsigai	Cold	-0,149838365	-1,825817234
HUTSI 3	Hu Tsigai	Cold	-0,217071297	-0,517236185
HUTSI 4	Hu Tsigai	Cold	-0,645021568	0,494846761
HUTSI 5	Hu Tsigai	Cold	-0,449967869	0,369679543
HUTSI 6	Hu Tsigai	Cold	-0,515200288	-1,084321216
HUTSI 7	Hu Tsigai	Cold	-0,828095855	0,43573812
HUTSI 8	Hu Tsigai	Cold	-0,317354116	-1,73789489
HUTSI 9	Hu Tsigai	Cold	0,184515159	-2,218671358
HUTSI 10	Hu Tsigai	Cold	4,382818935	-0,482976244
HUTSI 11	Hu Tsigai	Cold	-0,22307114	-0,176310893
HUTSI 12	Hu Tsigai	Cold	-0,06893651	-0,551411695
HUTSI 13	Hu Tsigai	Cold	-0,713218386	1,279517368
HUTSI 14	Hu Tsigai	Cold	4,146673707	1,612657223
HUTSI 15	Hu Tsigai	Cold	4,420291482	-0,439113137
HUTSI 16	Hu Tsigai	Cold	3,611876986	1,32076852
HUTSI 17	Hu Tsigai	Cold	-1,099530545	1,684816856
HUTSI 18	Hu Tsigai	Cold	-1,624318396	1,093358567
HUTSI 19	Hu Tsigai	Cold	-1,609853837	1,786219789
HUTSI 20	Hu Tsigai	Cold	0,135076707	-1,203644654
HUTSI 21	Hu Tsigai	Cold	-1,625058831	1,255539098
HUTSI 22	Hu Tsigai	Cold	4,226562382	-0,152153562
HUTSI 23	Hu Tsigai	Cold	-1,229492402	1,634872424
HUTSI 24	Hu Tsigai	Cold	-0,527070294	-0,065727794
HUTSI 25	Hu Tsigai	Cold	-0,148254023	-0,431266836
HUTSI 26	Hu Tsigai	Cold	0,089804352	-1,535641024
HUTSI 27	Hu Tsigai	Cold	-0,63232231	0,341695912
HUTSI 28	Hu Tsigai	Cold	-0,709669823	-0,63518111
HUTSI 29	Hu Tsigai	Cold	-1,586007913	2,198943572
HUTSI 30	Hu Tsigai	Cold	4,801689839	0,675504591
HUTSI 31	Hu Tsigai	Cold	-0,372876952	-1,907265723
HUTSI 32	Hu Tsigai	Cold	-0,855189141	1,386124151
HUTSI 33	Hu Tsigai	Cold	1,404708765	0,747402334
HURAC 1	Hu Racka	Cold	0,185354234	-1,909187228
HURAC 2	Hu Racka	Cold	0,228676964	-1,916489468
HURAC 3	Hu Racka	Cold	0,478645241	-3,917141546
HURAC 4	Hu Racka	Cold	0,150203641	-3,103712665
HURAC 5	Hu Racka	Cold	0,069194042	0,094016486
HURAC 6	Hu Racka	Cold	-1,147372869	1,279703379
HURAC 7	Hu Racka	Cold	-1,235937733	0,727952999
HURAC 8	Hu Racka	Cold	0,699544689	-4,149314202
HURAC 9	Hu Racka	Cold	0,915643683	-3,130626098
HURAC 10	Hu Racka	Cold	-0,28390498	-1,028771813
HURAC 11	Hu Racka	Cold	-0,878354023	0,507806273
HURAC 12	Hu Racka	Cold	0,372842823	-1,392797787
HURAC 13	Hu Racka	Cold	-0,035525545	-0,81527037
HURAC 14	Hu Racka	Cold	0,686088248	-2,79057066
HURAC 15	Hu Racka	Cold	-0,744199621	-0,599177549
HURAC 16	Hu Racka	Cold	-0,221903864	-0,230813373

HURAC 17	Hu Racka	Cold	-0,194526336	-2,621934999
HURAC 18	Hu Racka	Cold	-0,176504562	-1,808706373
HURAC 19	Hu Racka	Cold	-0,363938357	-2,076709997
HURAC 20	Hu Racka	Cold	-0,377000511	-1,449267162
HURAC 21	Hu Racka	Cold	-0,04922732	-2,458485584
HURAC 22	Hu Racka	Cold	-0,574811278	0,18906396
HURAC 23	Hu Racka	Cold	0,416988848	-2,183981684
HURAC 24	Hu Racka	Cold	-1,050978723	-0,959051675
HURAC 25	Hu Racka	Cold	-0,374677633	-0,034169861
HURAC 26	Hu Racka	Cold	0,487916674	-3,064341089
HURAC 27	Hu Racka	Cold	-0,67158014	0,753368898
HURAC 28	Hu Racka	Cold	0,021456993	-1,585226161
HURAC 29	Hu Racka	Cold	0,125322377	-2,283407249
HURAC 30	Hu Racka	Cold	-1,063201005	1,396920787
HURAC 31	Hu Racka	Cold	-1,237418604	1,052314062
HURAC 32	Hu Racka	Cold	0,352893733	-2,001122698
HURAC 33	Hu Racka	Cold	-0,26193426	-0,995101205
HURAC 34	Hu Racka	Cold	-0,335301882	-0,980830142
HURAC 35	Hu Racka	Cold	0,917027938	-3,09778358
HURAC 36	Hu Racka	Cold	0,535380706	-3,329916659
HURAC 37	Hu Racka	Cold	-0,307575988	-1,522589736
HURAC 38	Hu Racka	Cold	0,475533614	-1,905031733
HURAC 39	Hu Racka	Cold	0,221014184	-1,308382839
HURAC 40	Hu Racka	Cold	4,225314671	-0,277106593
HURAC 41	Hu Racka	Cold	-0,514306155	-0,879025973
HURAC 42	Hu Racka	Cold	-0,410254422	-2,537123597
HURAC 43	Hu Racka	Cold	0,929309651	-3,87507088
HURAC 44	Hu Racka	Cold	0,042299286	-2,55545733
HURAC 45	Hu Racka	Cold	0,198360381	-1,424695469
HURAC 46	Hu Racka	Cold	3,558546639	0,515615897
HURAC 47	Hu Racka	Cold	4,685143357	-0,677725162
HURAC 48	Hu Racka	Cold	0,607701913	-3,218543808
KAR 1	Botosani Karakul	Hot eu	-0,670812276	0,15636841
KAR 2	Botosani Karakul	Hot eu	-0,477182888	-0,510151517
KAR 3	Botosani Karakul	Hot eu	-0,219137713	-0,125435062
KAR 4	Botosani Karakul	Hot eu	-0,624385476	1,883791992
KAR 5	Botosani Karakul	Hot eu	-0,128451554	0,406342563
KAR 6	Botosani Karakul	Hot eu	0,001104103	0,138238393
KAR 7	Botosani Karakul	Hot eu	-0,099398364	-0,045647706
KAR 8	Botosani Karakul	Hot eu	1,367108551	0,742860406
KAR 9	Botosani Karakul	Hot eu	-0,033198054	0,126041377
KAR 10	Botosani Karakul	Hot eu	-0,459009265	0,508253766
KAR 11	Botosani Karakul	Hot eu	-1,409548332	2,674303579
KAR 12	Botosani Karakul	Hot eu	2,17142029	1,408994217
KAR 13	Botosani Karakul	Hot eu	-1,636926333	2,930619289
KAR 14	Botosani Karakul	Hot eu	0,02909085	0,04486201
KAR 15	Botosani Karakul	Hot eu	-0,136054357	0,150287422
KAR 16	Botosani Karakul	Hot eu	3,874913979	1,672080222
KAR 17	Botosani Karakul	Hot eu	-0,115863349	0,844204636
KAR 18	Botosani Karakul	Hot eu	-0,638347343	0,305416584
KAR 19	Botosani Karakul	Hot eu	-0,098957525	0,149200765
KAR 20	Botosani Karakul	Hot eu	-0,526609598	0,631489254
KAR 21	Botosani Karakul	Hot eu	-0,646009027	0,682785561
KAR 22	Botosani Karakul	Hot eu	-0,665723243	0,151408427
KAR 23	Botosani Karakul	Hot eu	-0,779066025	0,27126743
KAR 24	Botosani Karakul	Hot eu	-0,874840714	0,182846628
KAR 25	Botosani Karakul	Hot eu	4,666608833	0,686324656
RORAC 1	Ro Racka	Cold	-0,347104952	-0,535626334
RORAC 2	Ro Racka	Cold	-0,644448046	-1,435204616
RORAC 3	Ro Racka	Cold	4,407418158	-0,117237859
RORAC 4	Ro Racka	Cold	4,493700692	0,626482169
RORAC 5	Ro Racka	Cold	-0,067491296	-0,741621522
RORAC 6	Ro Racka	Cold	1,008513109	-3,369050449
RORAC 7	Ro Racka	Cold	0,617154135	-2,85910089

RORAC 8	Ro Racka	Cold	-0,380551505	-0,14434177
RORAC 9	Ro Racka	Cold	-0,056437293	-1,793725013
RORAC 10	Ro Racka	Cold	-1,245476558	0,697461523
RORAC 11	Ro Racka	Cold	0,534640566	-1,431059708
RORAC 12	Ro Racka	Cold	-0,285061064	-0,006568354
RORAC 13	Ro Racka	Cold	0,971055617	-2,738568709
RORAC 14	Ro Racka	Cold	3,76332053	1,685721053
RORAC 15	Ro Racka	Cold	0,66193521	-2,218248946
RORAC 16	Ro Racka	Cold	0,142969908	-1,629350156
RORAC 17	Ro Racka	Cold	4,72405749	-0,384961909
RORAC 18	Ro Racka	Cold	-0,280042369	-0,936935346
RORAC 19	Ro Racka	Cold	0,385854671	-2,851287854
RORAC 20	Ro Racka	Cold	0,316017433	-1,650732184
RORAC 21	Ro Racka	Cold	5,013324135	-1,008090406
RORAC 22	Ro Racka	Cold	0,407592894	-0,726095989
RORAC 23	Ro Racka	Cold	0,381464603	-1,855846591
RORAC 24	Ro Racka	Cold	-0,461498373	0,550102216
RORAC 25	Ro Racka	Cold	-0,227612407	-1,07785492
RORAC 26	Ro Racka	Cold	4,857657625	-0,071420911
RORAC 27	Ro Racka	Cold	4,370061824	1,344674427
RORAC 28	Ro Racka	Cold	-0,485943016	-0,573145094
RORAC 29	Ro Racka	Cold	-1,099743999	0,487556007
RORAC 30	Ro Racka	Cold	-0,183520434	-1,19423783
RORAC 31	Ro Racka	Cold	0,649901819	-2,521430764
RORAC 32	Ro Racka	Cold	-0,065862158	-0,598971529
RORAC 33	Ro Racka	Cold	3,651853504	2,235983227
RORAC 34	Ro Racka	Cold	-0,327134593	-0,417081098
RORAC 35	Ro Racka	Cold	0,334592751	-1,61706202
RORAC 36	Ro Racka	Cold	-0,414110331	0,043005901
RORAC 37	Ro Racka	Cold	-0,053448771	-1,432048713
RORAC 38	Ro Racka	Cold	-0,207425348	-0,928248158
RORAC 39	Ro Racka	Cold	0,501119917	-3,464484257
RORAC 40	Ro Racka	Cold	-0,304985316	-0,308100243
RORAC 41	Ro Racka	Cold	-0,594348528	0,518309038
RORAC 42	Ro Racka	Cold	0,534655275	-3,564247996
RORAC 43	Ro Racka	Cold	-0,156422952	-0,574505097
RORAC 44	Ro Racka	Cold	-0,55484227	-0,316473473
RORAC 45	Ro Racka	Cold	-5,592730658	-3,023460907
RORAC 46	Ro Racka	Cold	-5,579203192	-3,462032349
RORAC 47	Ro Racka	Cold	4,28675051	-0,26408696
RORAC 48	Ro Racka	Cold	-0,035613889	-1,236198017
RORAC 49	Ro Racka	Cold	-0,014874825	-1,27581454
RORAC 50	Ro Racka	Cold	-0,459481722	0,389893385
RORAC 51	Ro Racka	Cold	-0,302870122	-1,035289405
RORAC 52	Ro Racka	Cold	-0,432084855	1,211411827
RORAC 53	Ro Racka	Cold	-0,269420674	-0,286801061
RORAC 54	Ro Racka	Cold	3,859835649	1,025883076
RORAC 55	Ro Racka	Cold	-0,591263576	0,355835093
RORAC 56	Ro Racka	Cold	-0,345430635	1,138136897
RORAC 57	Ro Racka	Cold	3,547367608	0,993971242
RORAC 58	Ro Racka	Cold	-0,719767705	0,657841307
RORAC 59	Ro Racka	Cold	0,441455665	-2,879346552
RORAC 60	Ro Racka	Cold	-0,273613897	-0,346292976
RORAC 61	Ro Racka	Cold	-0,291937606	-1,544005526
RORAC 62	Ro Racka	Cold	-0,45420155	0,062770588
TRANSMER 1	Transylvanian Merino	Hot eu	-0,415007037	0,691339851
TRANSMER 2	Transylvanian Merino	Hot eu	0,331307306	-0,887075937
TRANSMER 3	Transylvanian Merino	Hot eu	-0,740918842	0,996175004
TRANSMER 4	Transylvanian Merino	Hot eu	-0,47859282	0,192070989
TRANSMER 5	Transylvanian Merino	Hot eu	-0,624413154	0,951684775
TRANSMER 6	Transylvanian Merino	Hot eu	-0,32580233	0,49778289
TRANSMER 7	Transylvanian Merino	Hot eu	-0,6835881	0,645453314
TRANSMER 8	Transylvanian Merino	Hot eu	-0,334185129	-0,132173324
TRANSMER 9	Transylvanian Merino	Hot eu	2,064362194	0,862139031

TRANSMER 10	Transylvanian Merino	Hot eu	-0,852487622	1,771159205
TRANSMER 11	Transylvanian Merino	Hot eu	-0,006862559	0,139241352
TRANSMER 12	Transylvanian Merino	Hot eu	-0,774878794	1,172907249
TRANSMER 13	Transylvanian Merino	Hot eu	-0,551239406	0,883706402
TRANSMER 14	Transylvanian Merino	Hot eu	-0,524736538	0,733088433
TRANSMER 15	Transylvanian Merino	Hot eu	-0,72216199	0,499467009
TRANSMER 16	Transylvanian Merino	Hot eu	-1,15176587	0,845170408
TRANSMER 17	Transylvanian Merino	Hot eu	-1,384488589	1,842425232
TRANSMER 18	Transylvanian Merino	Hot eu	-1,347397399	1,683281264
TRANSMER 19	Transylvanian Merino	Hot eu	-0,122510635	-0,798648249
TRANSMER 20	Transylvanian Merino	Hot eu	-0,716505279	1,126307928
TRANSMER 21	Transylvanian Merino	Hot eu	0,153601063	-1,65113218
TRANSMER 22	Transylvanian Merino	Hot eu	-0,998134352	1,571828586
TRANSMER 23	Transylvanian Merino	Hot eu	0,073233704	0,088817218
TRANSMER 24	Transylvanian Merino	Hot eu	-0,093005224	1,672252395
TRANSMER 25	Transylvanian Merino	Hot eu	-0,921998863	0,855128088
TRANSMER 26	Transylvanian Merino	Hot eu	-0,105415184	-0,442063746
TRANSMER 27	Transylvanian Merino	Hot eu	0,108828154	-1,050606289
TRANSMER 28	Transylvanian Merino	Hot eu	-0,47613951	0,212121284
TRANSMER 29	Transylvanian Merino	Hot eu	-1,145891998	0,955342316
TRANSMER 30	Transylvanian Merino	Hot eu	3,832514274	2,137819836
TRANSMER 31	Transylvanian Merino	Hot eu	-1,065327336	0,714456496
TRANSMER 32	Transylvanian Merino	Hot eu	-1,097401154	0,914353591
TRANSMER 33	Transylvanian Merino	Hot eu	0,088344131	-0,128906056
TRANSMER 34	Transylvanian Merino	Hot eu	-0,46445577	1,475314612
TRANSMER 35	Transylvanian Merino	Hot eu	-0,485329498	1,454078065
TRANSMER 36	Transylvanian Merino	Hot eu	-0,07784396	-0,148795272
TRANSMER 37	Transylvanian Merino	Hot eu	-0,498971043	0,39455937
TRANSMER 38	Transylvanian Merino	Hot eu	-0,358992003	-0,06503116
TRANSMER 39	Transylvanian Merino	Hot eu	-0,563652793	1,716783586
TRANSMER 40	Transylvanian Merino	Hot eu	-1,038961047	2,485998116
TRANSMER 41	Transylvanian Merino	Hot eu	-0,63896602	0,036900761
TRANSMER 42	Transylvanian Merino	Hot eu	-0,474019236	1,075639312
TRANSMER 43	Transylvanian Merino	Hot eu	-1,073405788	2,230550101
TRANSMER 44	Transylvanian Merino	Hot eu	-0,453509628	-0,265622555
TRANSMER 45	Transylvanian Merino	Hot eu	0,053004466	-0,314028595
ROTSI 1	Romanian Tsigai	Hot eu	-0,21788679	0,214649083
ROTSI 2	Romanian Tsigai	Hot eu	0,605830021	-0,927709885
ROTSI 3	Romanian Tsigai	Hot eu	0,461664162	-1,451401566
ROTSI 4	Romanian Tsigai	Hot eu	-1,34333538	1,281709317
ROTSI 5	Romanian Tsigai	Hot eu	-1,058828117	0,973500099
ROTSI 6	Romanian Tsigai	Hot eu	-0,47736903	0,395547019
ROTSI 7	Romanian Tsigai	Hot eu	-0,553831602	0,260437537
ROTSI 8	Romanian Tsigai	Hot eu	-0,355957359	-0,695404586
ROTSI 9	Romanian Tsigai	Hot eu	-0,071076639	-0,682747909
ROTSI 10	Romanian Tsigai	Hot eu	-0,11250734	-0,345220768
ROTSI 11	Romanian Tsigai	Hot eu	-0,601110547	1,398245737
ROTSI 12	Romanian Tsigai	Hot eu	-0,303944061	0,762468847
ROTSI 13	Romanian Tsigai	Hot eu	-0,249188665	-1,445787239
ROTSI 14	Romanian Tsigai	Hot eu	0,085848212	-0,537706688
ROTSI 15	Romanian Tsigai	Hot eu	-0,058020424	-0,767785208
ROTSI 16	Romanian Tsigai	Hot eu	0,439777519	-1,50541487
ROTSI 17	Romanian Tsigai	Hot eu	0,073859114	-0,816403165
ROTSI 18	Romanian Tsigai	Hot eu	-1,164655554	1,817381576
ROTSI 19	Romanian Tsigai	Hot eu	-0,372320249	-1,385144465
ROTSI 20	Romanian Tsigai	Hot eu	-0,230647121	0,451189566
TUR 1	Turcana	Cold	-0,896042022	1,685007523
TUR 2	Turcana	Cold	4,774252127	0,169986219
TUR 3	Turcana	Cold	-0,810796874	0,799010691
TUR 4	Turcana	Cold	-0,174036217	-0,642759301
TUR 5	Turcana	Cold	-0,335380491	-1,309241304
TUR 6	Turcana	Cold	-1,327464339	0,824924745
TUR 7	Turcana	Cold	-0,151514892	-0,391827875
TUR 8	Turcana	Cold	0,745379451	-2,120763989

TUR 9	Turcana	Cold	-0,447686272	0,402497845
TUR 10	Turcana	Cold	3,971850643	0,423256184
TUR 11	Turcana	Cold	-1,036756466	0,859732602
TUR 12	Turcana	Cold	3,622827685	0,589565201
TUR 13	Turcana	Cold	-1,00611914	1,044501026
TUR 14	Turcana	Cold	-0,916991373	1,287251604
TUR 15	Turcana	Cold	3,450041923	1,872011548
TUR 16	Turcana	Cold	-0,716838799	-0,130498502
TUR 17	Turcana	Cold	3,294597997	3,185163422
TUR 18	Turcana	Cold	0,167450397	-1,027045554
TUR 19	Turcana	Cold	-0,344992685	0,505611987
TUR 20	Turcana	Cold	-1,270015682	-0,24636182
TUR 21	Turcana	Cold	0,177949796	-0,60444532
TUR 22	Turcana	Cold	0,357018318	-1,460430058
TUR 23	Turcana	Cold	0,214745911	-2,301229223
TUR 24	Turcana	Cold	-0,529113869	0,122063084
TUR 25	Turcana	Cold	3,858233242	2,639727095
TUR 26	Turcana	Cold	-1,221780222	1,030898272
TUR 27	Turcana	Cold	-0,219760975	-0,634747015
TUR 28	Turcana	Cold	-0,098578225	0,656329186
TUR 29	Turcana	Cold	-0,752286558	0,413690635
TUR 30	Turcana	Cold	0,733882282	-2,840927581
TUR 31	Turcana	Cold	0,634918288	-2,462306339
TUR 32	Turcana	Cold	-0,376098257	-0,94128223
TUR 33	Turcana	Cold	-0,43503842	0,27822267
TUR 34	Turcana	Cold	4,120064663	-0,023471536
TUR 35	Turcana	Cold	-0,081434219	-0,94181208
TUR 36	Turcana	Cold	-0,400023383	0,946034267
TUR 37	Turcana	Cold	0,5352087	-2,382704762
TUR 38	Turcana	Cold	0,084523063	-1,20479617
TUR 39	Turcana	Cold	-0,666776971	-1,05832453
TUR 40	Turcana	Cold	0,422135557	-1,454361821
TUR 41	Turcana	Cold	0,877969592	-2,901261143
TUR 42	Turcana	Cold	0,53372783	-2,0583437
TUR 43	Turcana	Cold	-0,961155627	1,024820467
TUR 44	Turcana	Cold	-0,093344168	-0,716142164
TUR 45	Turcana	Cold	-0,256444621	-0,284221173
TUR 46	Turcana	Cold	-0,989220496	0,623832987
TUR 47	Turcana	Cold	0,210983267	-0,08261464
TUR 48	Turcana	Cold	-0,691355062	1,569550939
TUR 49	Turcana	Cold	0,767960231	-1,866612762
TUR 50	Turcana	Cold	0,393472269	-1,651671457
TUR 51	Turcana	Cold	0,910471793	-3,032637691
TUR 52	Turcana	Cold	0,174568798	-0,977406853
TUR 53	Turcana	Cold	-0,795405757	1,418739444
TUR 54	Turcana	Cold	0,277466491	-0,681111002
TUR 55	Turcana	Cold	-0,521944372	-0,065427159
TUR 56	Turcana	Cold	0,030036147	-0,78223173
TUR 57	Turcana	Cold	-0,284641846	0,127188993
TUR 58	Turcana	Cold	-0,752234469	0,573560328
HUMER 1	Hungarian Merino	Cold	-1,390261423	1,523169889
HUMER 2	Hungarian Merino	Cold	-0,827508541	1,823640722
HUMER 3	Hungarian Merino	Cold	-0,783600642	2,016105919
HUMER 4	Hungarian Merino	Cold	0,539663303	-1,109801869
HUMER 5	Hungarian Merino	Cold	-0,624239549	-0,007259629
HUMER 6	Hungarian Merino	Cold	-1,380815935	2,251255556
HUMER 7	Hungarian Merino	Cold	-1,19680114	1,083379789
HUMER 8	Hungarian Merino	Cold	-1,031706494	2,422463168
HUMER 9	Hungarian Merino	Cold	-1,866209573	2,743502179
HUMER 10	Hungarian Merino	Cold	-0,369928964	1,592033536
HUMER 11	Hungarian Merino	Cold	-0,436519291	0,602583733
HUMER 12	Hungarian Merino	Cold	-0,146399431	0,515508718
HUMER 13	Hungarian Merino	Cold	-0,354416932	0,386786949
HUMER 14	Hungarian Merino	Cold	-0,993774507	1,882345146

HUMER 15	Hungarian Merino	Cold	-1,246530378	2,206367882
HUMER 16	Hungarian Merino	Cold	-1,128013322	1,506262653
HUMER 17	Hungarian Merino	Cold	-0,795755658	1,306209135
HUMER 18	Hungarian Merino	Cold	-1,771266903	2,779258822
HUMER 19	Hungarian Merino	Cold	-0,796130366	1,231727971
HUMER 20	Hungarian Merino	Cold	-1,023538967	1,489478064
HUMER 21	Hungarian Merino	Cold	-0,47859282	0,192070989
HUMER 22	Hungarian Merino	Cold	-1,19680114	1,083379789
HUMER 23	Hungarian Merino	Cold	-0,284896501	0,393600393
HUMER 24	Hungarian Merino	Cold	-1,662168233	4,019715057
HUMER 25	Hungarian Merino	Cold	-1,325791863	3,288098514
HUMER 26	Hungarian Merino	Cold	0,54228565	-1,342837286
HUMER 27	Hungarian Merino	Cold	0,098679882	-0,690410795
HUMER 28	Hungarian Merino	Cold	-1,606494792	2,395779881
HUMER 29	Hungarian Merino	Cold	-1,341616368	3,220466242
HUMER 30	Hungarian Merino	Cold	-1,530887306	2,06927735
HUMER 31	Hungarian Merino	Cold	-1,144756678	1,959108687
HUMER 32	Hungarian Merino	Cold	-0,530121559	1,278982012
HUMER 33	Hungarian Merino	Cold	-1,04967354	0,958349345
HUMER 34	Hungarian Merino	Cold	-0,477415987	1,622218554
HUMER 35	Hungarian Merino	Cold	0,041802907	-0,096694934
AWAS 1	Hungarian Awassi	Hot eu	3,827354908	1,344662327
AWAS 2	Hungarian Awassi	Hot eu	0,030036147	-0,78223173
AWAS 3	Hungarian Awassi	Hot eu	-0,424105904	-0,230493451
AWAS 4	Hungarian Awassi	Hot eu	-1,589259748	2,750170081
AWAS 5	Hungarian Awassi	Hot eu	-1,02538663	2,53270593
AWAS 6	Hungarian Awassi	Hot eu	-6,741431453	-0,881073826
AWAS 7	Hungarian Awassi	Hot eu	3,493304333	2,263726458
AWAS 8	Hungarian Awassi	Hot eu	0,238952448	-0,351735066
AWAS 9	Hungarian Awassi	Hot eu	3,534213786	1,989228957
AWAS 10	Hungarian Awassi	Hot eu	-0,63772558	0,605606936
AWAS 11	Hungarian Awassi	Hot eu	-0,584969109	1,507972531
AWAS 12	Hungarian Awassi	Hot eu	4,31103742	1,310269626
AWAS 13	Hungarian Awassi	Hot eu	-1,337261535	2,337407237
AWAS 14	Hungarian Awassi	Hot eu	-0,289024899	-0,241313516
AWAS 15	Hungarian Awassi	Hot eu	0,640274762	-0,67226187
AWAS 16	Hungarian Awassi	Hot eu	-0,436519291	0,602583733
AWAS 17	Hungarian Awassi	Hot eu	0,656739163	-1,055747358
AWAS 18	Hungarian Awassi	Hot eu	-6,477386645	-1,40804705
AWAS 19	Hungarian Awassi	Hot eu	-0,38458325	0,654205467
AWAS 20	Hungarian Awassi	Hot eu	-1,00611914	1,044501026
AWAS 21	Hungarian Awassi	Hot eu	-1,071877965	2,391468295
AWAS 22	Hungarian Awassi	Hot eu	-1,316680089	2,134044694
AWAS 23	Hungarian Awassi	Hot eu	0,316017433	-1,650732184
AWAS 24	Hungarian Awassi	Hot eu	-0,724114122	1,72971893
AWAS 25	Hungarian Awassi	Hot eu	3,770273043	1,697082088
AWAS 26	Hungarian Awassi	Hot eu	-0,609256019	-0,066384055
AWAS 27	Hungarian Awassi	Hot eu	-0,508283925	0,564729866
AWAS 28	Hungarian Awassi	Hot eu	-0,764699945	1,246767819
AWAS 29	Hungarian Awassi	Hot eu	3,88411728	2,207922877
AWAS 30	Hungarian Awassi	Hot eu	-5,757452711	-3,987348395
AWAS 31	Hungarian Awassi	Hot eu	0,595264297	-1,137860568
AWAS 32	Hungarian Awassi	Hot eu	-0,078662481	0,502599702
AWAS 33	Hungarian Awassi	Hot eu	4,097008984	1,128241949
AWAS 34	Hungarian Awassi	Hot eu	0,306226394	-1,050690417
AWAS 35	Hungarian Awassi	Hot eu	-1,329906793	2,123218083
AWAS 36	Hungarian Awassi	Hot eu	0,144215077	-0,662584744
AWAS 37	Hungarian Awassi	Hot eu	-1,315199218	1,809683631
AWAS 38	Hungarian Awassi	Hot eu	3,414287886	2,554614605
AWAS 39	Hungarian Awassi	Hot eu	-0,508562049	0,280120548
AWAS 40	Hungarian Awassi	Hot eu	-0,662607615	1,359946756

Appendix Table S5. A year-round analysis of the average and standard deviation of CT values for *IL10, TLR2, TLR4, TLR8*, and *HSP70* in Indigenous Tsigai, Merino, and White Dorper sheep from Hungary

Gene	Season	CT-Value (Mean± Std. Deviation)			
		Hungarian Tsigai	Hungarian Merino	White Dorper	Overall
HSP70	Summer	22.540±0.473	22.500±0.279	22.500±0.628	22.516±0.482^{a,b}
	Autumn	24.835±1.032	24.413±1.260	23.940±0.758	24.393±0.992^{c}
	Winter	22.582±1.040	22.273±0.055	21.515±0.597	22.093±0.875^{a}
	Spring	23.037±0.934	23.203±0.106	22.898±0.691	23.015±0.705^{b}
	Overall	23.248±1.273^{a}	23.097±1.031^{a}	23.7133±1.086^{a}	23.004±1.162
IL10	Summer	32.953±1.581	32.897±1.055	32.122±1.946	32.609±1.605^{c}
	Autumn	31.215±0.757	30.127±0.624	30.345±0.441	30.649±0.752^{b}
	Winter	29.808±1.496	28.503±0.055	28.175±0.444	28.894±1.218^{a}
	Spring	29.755±0.981	29.473±0.352	29.110±0.527	29.441±0.742^{a}
	Overall	30.933±1.772^{b}	30.250±1.791^{a,b}	29.938±1.802^{a}	30.398±1.815
TLR2	Summer	26.927±1.259	26.400±1.003	26.115±1.283	26.497±1.201^{b}
	Autumn	27.528±1.370	25.370±0.491	25.698±0.549	26.365±1.340^{b}
	Winter	24.195±1.214	24.313±1.270	23.837±0.243	24.075±0.906^{a}
	Spring	24.453±0.778	23.843±0.167	24.095±0.807	24.188±0.716^{a}
	Overall	25.759±2.023^{b}	24.982±1.261^{a,b}	24.936±1.262^{a}	25.281±1.559
TLR4	Summer	24.617±0.731	24.363±0.548	23.002±0.660	23.920±1.000^{a}
	Autumn	26.613±1.076	25.640±1.521	25.720±0.620	26.061±1.049^{b}
	Winter	23.613±0.275	23.787±1.278	22.983±0.451	23.396±0.677^{a}
	Spring	24.105±0.767	23.837±0.317	23.095±0.339	23.647±0.703^{a}
	Overall	24.737±1.367^{b}	24.401±1.183^{b}	23.700±1.292^{a}	24.256±1.365
TLR8	Summer	26.572±1.185	25.910±1.404	25.873±1.529	26.160±1.319^{c}
	Autumn	25.952±0.905	24.820±1.129	24.340±0.919	25.081±1.162^{b}
	Winter	23.610±1.528	23.620±1.652	23.048±0.557	23.387±1.190^{a}
	Spring	23.282±0.707	22.953±0.826	22.562±0.237	22.928±0.638^{a}
	Overall	24.854±1.798^{b}	24.326±1.616^{a,b}	23.956±1.578^{a}	24.389±1.698
GAPDH	Summer	26.590±2.218	25.013±2.806	25.983±3.166	26.032±2.616
	Autumn	22.877±1.181	21.450±0.236	21.902±0.387	22.201±0.956
	Winter	21.833±1.724	21.787±1.180	21.107±1.462	21.533±1.467
	Spring	21.662±0,707	21.710±0.295	21.640±0.664	21.663±0.591
	Overall	23.240±2.501	22.490±2.011	22.658±2.589	22.857±2.589

SD: Standard deviation; $^{a\text{-}c}$: different superscript showed significant difference ($p< 0.05$)

Appendix Table S6. CT value gene expression of each samples in four different seasons

		CT Value AUTUMN 2020 (19/11/20 12.00-13.30)					CT Value Summer 2019 (13/08/19 12.00-13.00)					CT Value winter 2020 (22/01/20 12.00-13.30)					CT Value spring 2020 (29/04/20 12.00-13.30)				
Breed	Sex	IL10	TLR2	TLR4	TLR8	HSP70	IL10	TLR2	TLR4	TLR8	HSP70	IL10	TLR2	TLR4	TLR8	HSP70	IL10	TLR2	TLR4	TLR8	HSP70
Merino	Ram	30.42	25.81	26.84	25.79	25.2	33.25	26.55	23.93	25.39	22.19	28.56	25.12	25.26	25.22	22.3	29.26	23.94	23.69	23.43	23.30
Merino	Ewes	30.55	25.46	26.15	25.09	25.08	33.73	27.32	24.18	27.5	22.73	28.45	22.85	22.97	21.92	22.21	29.28	23.94	23.62	23.43	23.09
Merino	Ewes	29.41	24.84	23.93	23.58	22.96	31.71	25.33	24.98	24.84	22.58	28.5	24.97	23.13	23.72	22.31	29.88	23.65	24.20	22.00	23.22
Tsigai	Ewes	30.91	25.53	25.68	25.12	24.18	33.99	27.24	24.02	26.58	21.85	31.79	22.97	23.58	22.13	22.87	28.77	23.70	23.30	22.24	22.10
Tsigai	Ewes	32.01	28.61	27.83	25.83	24.45	34.42	27.55	24.09	26.41	22.03	28.98	24.31	23.54	23.77	20.93	29.56	24.56	23.92	23.40	22.33
Tsigai	Ewes	31.29	27.26	26.62	26.19	24.99	34.18	28.8	25.6	28.21	22.71	30.04	24.46	23.65	23.43	22.88	29.80	24.12	23.93	23.48	23.24
Tsigai	Ram	30.18	28.8	25.22	24.98	24.17	32.74	26.65	24.56	27.30	22.87	28.22	23.2	23.43	22.78	23.11	31.48	25.94	25.54	24.34	24.74
Tsigai	Ram	30.76	26.36	26.5	26.1	24.37	30.32	25.09	23.99	24.65	22.89	28.5	23.87	23.35	23.04	21.83	30.03	24.20	24.23	23.42	22.96
Tsigai	Ram	32.14	28.61	27.83	27.49	26.85	32.07	26.23	25.44	26.28	22.89	31.32	26.36	24.13	26.51	23.87	28.89	24.20	23.71	22.81	22.85
White Dorper	Ewes	30.49	25.8	25.76	22.85	22.6	31.62	26.73	24.01	26.35	23.34	28.24	23.96	22.3	22.39	20.66	28.47	25.35	23.18	22.30	22.69
White Dorper	Ewes	30.05	25.89	25.89	25.46	24.55	30.63	24.1	23.07	24.04	22.66	28.46	23.84	23.27	22.86	21.11	29.49	23.96	23.01	22.56	22.60
White Dorper	Ewes	30.05	25.89	25.89	24.26	24.26	31.18	26.3	22.91	26.3	22.63	27.34	23.52	22.79	22.94	22.14	29.76	23.67	23.54	22.95	23.76
White Dorper	Ewes	30.91	25.53	25.68	25.14	24.66	30.35	25.49	22.95	24.49	22.83	28.54	23.69	23.34	23.17	22.21	29.47	24.58	22.73	22.46	23.72
White Dorper	Ram	30.76	26.36	26.5	24.03	23.92	33.65	26.12	23.13	25.74	21.61	28.06	23.78	23.47	22.87	21.56	28.70	24.03	23.39	22.71	22.04
White Dorper	Ram	29.81	24.72	24.6	24.3	23.65	35.3	27.95	21.94	28.32	21.93	28.41	24.23	22.73	24.06	21.41	28.77	22.98	22.72	22.39	22.58

Appendix Table S7. The 108 Differentially Expressed Genes (DEGs) between the black coated to white coated Hortobágyi Racka sheep skin

	ID	Mean Black (TPM)	Mean White (TPM)	FC	logFC	P.Value	adj.P.Val
1	*TYRP1*	519.97626	0.3832387	461.5543335	8.8503567	7.751E-08	0.0004038
2	*PMEL*	430.14116	2.9641293	131.2590994	7.0362736	1.928E-07	0.0005024
3	*TRPM1*	659.88162	31.332963	19.77828358	4.3058453	4.174E-08	0.0004038
4	*MLANA*	276.71117	15.08683	18.01753989	4.1713301	1.76E-07	0.0005024
5	*SLC24A5*	121.90469	4.0078804	25.88307106	4.6939369	6.608E-05	0.0635098
6	*SLC24A4*	31.549242	0.7782814	20.02833409	4.3239705	1.683E-05	0.021917
7	*SLC45A2*	169.87382	7.6221039	18.57059502	4.2149481	1.934E-06	0.0033581
8	*TYR*	93.160431	3.9810151	23.88651015	4.5781242	9.836E-05	0.0788407
9	*IRF4*	23.998514	0	21.63678725	4.4354144	9.624E-05	0.0788407
10	*DCT*	110.91448	7.5615248	13.33444594	3.737086	1.004E-05	0.0149424
11	*OCA2*	401.51407	32.772999	11.39922953	3.5108644	5.244E-07	0.0010928
12	*DDC*	127.38351	11.39467	9.692052796	3.2768023	2.239E-05	0.025927
13	*KRTAP6-1*	7.8147514	46.635245	-5.807034603	-2.5378016	0.0002584	0.1923504
14	*SNAP91*	34.215687	3.0406973	8.448393307	3.078677	0.0013683	0.5949236
15	*NR4A3*	19.811159	2.9526379	8.487018302	3.0852578	0.0014129	0.5949236
16	*PLXNC1*	167.8981	33.161242	5.301441467	2.4063847	6.704E-05	0.0635098
17	*LOC114113683*	48.716518	0	36.37784487	5.1849882	0.0104155	0.9999991
18	*LIPE*	120.57709	15.804497	6.522284621	2.7053774	0.00349	0.9999991
19	*LOC114110581*	1.9346241	21.901358	-8.050299506	-3.0090425	0.0044233	0.9999991
20	*FAM174B*	108.24458	26.61835	4.919482151	2.2985065	0.0010791	0.5622292
21	*PMFBP1*	1.8854171	16.712802	-7.38159727	-2.883933	0.0058743	0.9999991
22	*LOC101121371*	98.041377	42.56486	9.657734052	3.2716847	0.01017	0.9999991
23	*GPRIN3*	44.341048	9.0114523	4.304744405	2.1059276	0.0004964	0.3147518
24	*PRKG2*	49.192901	12.558622	4.827679258	2.2713298	0.0034235	0.9999991
25	*LOC114112897*	22.0544	0.2240013	12.63663772	3.6595407	0.0163772	0.9999991
26	*PPM1E*	23.160725	4.9910073	4.540456924	2.1828375	0.0056753	0.9999991
27	*LOC101106637*	28.229932	6.0125361	3.922304912	1.9717017	0.0022696	0.9095775
28	*AKR1B1*	41.708689	11.466239	3.893009189	1.9608858	0.0014274	0.5949236
29	*LOC101114011*	384.6274	91.786842	6.517671985	2.7043567	0.0176409	0.9999991
30	*LOC101116157*	94.66515	17.035414	4.563588861	2.1901688	0.0081358	0.9999991
31	*DLGAP1*	55.873102	14.3811	3.52732037	1.8185726	0.0053597	0.9999991
32	*PLXNB2*	3.2216123	14.918517	-3.943268788	-1.9793921	0.0118148	0.9999991
33	*KCNJ6*	2.8738136	14.914955	-4.980513898	-2.3162946	0.0194391	0.9999991
34	*RANGRF*	21.297045	6.634558	4.143915259	2.0509945	0.0119926	0.9999991

35	*GPNMB*	347.92802	101.21553	3.267331934	1.708113	0.0027611	0.9999991
36	*PAX3*	39.86624	9.9594345	3.479593957	1.798919	0.0058877	0.9999991
37	*ATF5*	149.11553	50.574717	3.020765634	1.5949143	0.0005135	0.3147518
38	*KHDRBS2*	2.2520736	15.975485	-5.497310635	-2.458726	0.025403	0.9999991
39	*LOC114118004*	344.29371	1198.6309	-3.501379562	-1.8079235	0.0080668	0.9999991
40	*VNN1*	27.410083	8.2027912	3.265537793	1.7073206	0.0034974	0.9999991
41	*LYPD1*	54.456811	18.227304	3.041102371	1.6045944	0.0013999	0.5949236
42	*RAB32*	27.505618	7.8159946	3.225660766	1.6895947	0.0039214	0.9999991
43	*KIT*	161.50455	55.036416	2.958042036	1.5646426	0.0003004	0.208697
44	*B4GALT6*	16.231174	4.1064936	3.936730512	1.976998	0.0203673	0.9999991
45	*CSRP2*	43.218061	14.896766	3.145540801	1.6533081	0.0076367	0.9999991
46	*NNAT*	29.614666	4.3834096	5.256139923	2.3940037	0.0315896	0.9999991
47	*PLP1*	19.310851	4.0836444	3.763322915	1.9120071	0.0221658	0.9999991
48	*CDK2*	88.629284	31.374215	2.762608661	1.4660312	0.0010097	0.5537608
49	*PAG3*	31.416273	3.7652148	4.972390316	2.3139395	0.0409713	0.9999991
50	*B3GALNT1*	42.447489	10.699727	3.508225656	1.8107415	0.022528	0.9999991
51	*FOS*	4.2725757	26.845072	-4.235115515	-2.0824013	0.0322067	0.9999991
52	*C11H17orf67*	20.307991	13.985509	5.198100217	2.3779844	0.0454676	0.9999991
53	*KRT1*	286.06975	885.7705	-3.664231566	-1.8735107	0.0284297	0.9999991
54	*CDK15*	30.788399	10.067277	3.012633679	1.5910253	0.0186719	0.9999991
55	*LOC101119050*	61.03794	23.271047	2.636132679	1.398423	0.0027776	0.9999991
56	*NUPR1*	131.46082	51.610788	2.554651187	1.3531263	0.0005496	0.3181487
57	*SLC31A2*	5.8977398	20.89055	-2.908606615	-1.5403282	0.0192583	0.9999991
58	*LOC101107153*	3.3595172	15.762439	-3.723253834	-1.896564	0.0481061	0.9999991
59	*CBX6*	64.938008	25.234726	2.537697572	1.3435201	0.0011936	0.5922313
60	*OSBPL7*	18.061979	6.0349824	2.853981987	1.5129762	0.0196542	0.9999991
61	*TBX3*	27.86095	11.102469	2.730561019	1.4491974	0.0175764	0.9999991
62	*OPCML*	4.8745408	15.8175	-2.838516037	-1.5051369	0.0220492	0.9999991
63	*LOC132659145*	56.116317	18.400177	2.769540127	1.4696464	0.0202387	0.9999991
64	*GRINA*	18.757577	6.5025437	2.791058277	1.4808122	0.0220952	0.9999991
65	*SERHL2*	76.66843	29.002005	2.577249587	1.3658323	0.0123583	0.9999991
66	*S100A13*	114.98293	43.719967	2.511996744	1.3288346	0.0041709	0.9999991
67	*SNAP25*	5.0409263	17.972976	-3.035786956	-1.6020705	0.0434756	0.9999991
68	*LOC101104027*	352.90251	1043.8879	-2.840868567	-1.5063321	0.0271013	0.9999991
69	*BACE2*	52.364026	20.436133	2.482640185	1.3118752	0.0042249	0.9999991
70	*PPP1R12C*	25.857378	9.1710475	2.566640048	1.359881	0.0141605	0.9999991
71	*LOC114118402*	6.5613546	14.512026	-2.856443846	-1.5142202	0.0353955	0.9999991
72	*CREM*	62.869299	26.787897	2.531434286	1.339955	0.0168364	0.9999991
73	*C15H11orf96*	58.961665	21.119182	2.797478316	1.4841269	0.0420539	0.9999991

74	*INKA2*	24.055548	9.9372122	2.604665007	1.3810978	0.0255763	0.9999991
75	*KBTBD6*	23.143888	8.665855	2.665370957	1.4143363	0.0336404	0.9999991
76	*RPL36*	163.70346	69.647441	2.440673724	1.2872794	0.0124084	0.9999991
77	*QDPR*	138.66007	63.560146	2.380673543	1.2513698	0.0095037	0.9999991
78	*CENPM*	16.926771	6.0468316	2.530216722	1.339261	0.0233135	0.9999991
79	*NOSIP*	32.071616	14.315774	2.567446495	1.3603342	0.0307445	0.9999991
80	*LOC106990092*	79.4073	31.535266	2.381904672	1.2521157	0.0133483	0.9999991
81	*VAT1L*	22.776256	7.9866782	2.513210856	1.3295317	0.0257171	0.9999991
82	*STK17B*	22.268452	55.899557	-2.335072026	-1.2234671	0.0100084	0.9999991
83	*SPTSSB*	66.629876	155.63873	-2.395811966	-1.2605147	0.0193313	0.9999991
84	*KCTD6*	13.006448	30.016883	-2.374808922	-1.2478114	0.0212415	0.9999991
85	*C1H3orf70*	8.2340579	21.104669	-2.425940095	-1.2785439	0.0334577	0.9999991
86	*SNX25*	63.461592	23.360357	2.427889768	1.2797029	0.042927	0.9999991
87	*SUSD6*	41.090851	96.253012	-2.249234265	-1.1694339	0.014364	0.9999991
88	*GALNT17*	25.24814	64.61166	-2.363795739	-1.2411054	0.0403656	0.9999991
89	*CD63*	384.95248	162.53862	2.277912484	1.1877123	0.0247626	0.9999991
90	*LGALS7*	70.456614	170.24827	-2.208799121	-1.1432622	0.0139409	0.9999991
91	*SIGLEC10*	25.360149	10.727074	2.270977917	1.1833137	0.0282976	0.9999991
92	*SHE*	25.585599	10.719319	2.295886322	1.1990512	0.0402111	0.9999991
93	*SLC7A5*	647.72129	303.89788	2.244073149	1.1661197	0.0266851	0.9999991
94	*FREM1*	12.634387	29.960532	-2.298324459	-1.2005825	0.0475215	0.9999991
95	*SRF*	110.18115	49.805594	2.262407466	1.1778588	0.0395082	0.9999991
96	*SIRPA*	87.006105	36.115735	2.240810836	1.1640209	0.0341884	0.9999991
97	*ZNF697*	29.635344	12.275168	2.210540196	1.144399	0.0494316	0.9999991
98	*SLC25A4*	146.75581	68.265564	2.124182451	1.0869077	0.0197991	0.9999991
99	*LOC101115044*	43.848272	20.515557	2.112293244	1.0788101	0.0352447	0.9999991
100	*REX1BD*	52.781041	25.033092	2.079695355	1.0563722	0.0291036	0.9999991
101	*POMT2*	39.20131	19.625524	2.111811263	1.0784809	0.0435008	0.9999991
102	*CA10*	46.859627	109.76067	-2.116079875	-1.0813941	0.0491246	0.9999991
103	*ARHGAP18*	45.04405	96.440044	-2.041874255	-1.029894	0.0209369	0.9999991
104	*LAMTOR4*	73.183851	37.281808	2.018449463	1.0132475	0.0324888	0.9999991
105	*PECAM1*	210.4449	109.48639	1.937310414	0.9540551	0.0146412	0.9999991
106	*PSMC5*	110.18242	59.761716	1.903941823	0.9289894	0.0438443	0.9999991
107	*NDUFS2*	219.76997	119.36936	1.875358021	0.907166	0.025331	0.9999991
108	*ATP5ME*	280.27876	154.71173	1.831768254	0.873237	0.0306325	0.9999991