Continental vs. tropical breed: immunity comparison under heat stress conditions utilizing qRT-PCR technique

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Abstract. Understanding the responses of animals to seasonal heat stress on the genomic level has led to the identification of genes implicated in thermal stress reaction mechanisms. In this study, the relative gene expression of Interleukin-10 (*IL-10*), an anti-inflammatory cytokine and biomarker for heat stress-mediated immune modulation, was observed during the summer and winter seasons in continental and tropical sheep breeds, namely, Hungarian Indigenous Tsigai and White Dorper, employing quantitative real-time polymerase chain reaction (qRT-PCR). Temperature-humidity indices (THI) were calculated to assess heat stress levels. The results indicate that *IL-10* CT-values were significantly higher during the summer, when heat stress prevails, in both sheep breeds compared to winter. While the White Dorper exhibited a higher numerical value for the summer relative gene expression ratio (16.2) compared to the Hungarian Indigenous Tsigai (12.3), no significant differences in CT values were observed between breeds or among sexes. These findings suggest the immune-adaptive characteristics of the two sheep breeds during seasonal heat stress. The variation of *IL-10* gene expression levels between the two breeds can be attributed to their geographical origins; the White Dorper emerging from arid subtropical South Africa and the Hungarian Indigenous Tsigai Sheep thriving in the seasonally harsh Carpathian climate for centuries.

Keywords: gene expression, heat stress, Hungarian Tsigai, *IL-10*, White Dorper

1 Introduction

As a major sector in the livestock industry, sheep production is key to ensuring global food security for the rapidly growing population. Its importance to low-income families all around the world as a source of food and livelihood cannot be overlooked as well [1]. With climate change projections pointing to worse scenarios of increased global temperatures in the years to come, it is essential to tailor-fit the livestock industry to a warmer and harsher environment by selecting animals with tolerance and adaptation traits [2]. Equipped with a robust knowledge of genes related to heat tolerance and adaptation, the livestock industry can keep up with the climatological challenges and sustain productivity.

Heat stress presents direct challenges to the well-being of animals that lead to an array of detrimental effects on their physiology, behavior, overall production performance, reproduction, and health [3]. In response to heat stress, the animal attempts to maintain homeostasis and re-establish homeothermy by invoking compensatory and adaptive mechanisms through behavioral and physiological changes to promote welfare and ensure survival [4].

The immune system of animals, in general, is one of the well-studied aspects of the physiological responses of livestock to heat stress. The multifarious interplay of hormones and key immune system cells and factors during heat stress leads to complex immune responses that involve rapid inflammatory reaction, antagonistic immunosuppression, and shifting of immune regimes, among others [5-6]. Understanding and knowing these mechanisms and the factors involved usher in the determination of reliable biological markers of heat response in animals with respect to immunity.

Cytokines are some of the most prominent actors in the immune system and, in this regard, the immune response to heat stress. Of them, the Interleukin 10 (*IL-10*) plays a major role in modulating the other cytokines and effectors of the immune system that attempts to preserve its integrity and function while facing heat stress [7-8].

A deeper investigation of genes associated with heat tolerance and adaptation in livestock has earned the attention of researchers in recent years because of the increasing availability of genomic tools and capabilities. Reverse transcription quantitative real-time polymerase chain reaction (RT-qPCR) is a reliable and key enabling technology for gene expression analysis. Its rapid, cost-effective, and robust approach in gene expression analysis has encouraged researchers to use it for many purposes, including the detection and measurement of the relative expression of genes associated with heat tolerance and adaptation [9].

The influence of seasons on the expression of several heat stress-related genes in livestock was investigated in dairy cattle [10-11], sheep [12], and pigs [13]. This study aims to bridge the scarcity of comparable studies in sheep that intend to investigate seasonal effects on the expression of heat tolerance genes such as *IL-10* as well as a comparison of tropical and temperate breeds on the same subject.

In the present study, the relative gene expression of *IL-10* was determined in the Hungarian Indigenous Tsigai

sheep and White Dorper sheep in Hungary during the summer and winter seasons. The Tsigai sheep is an indigenous temperate triple-purpose breed in the Central-Eastern European region that has been bred and maintained as part of the Hungarian sheep sector for more than two centuries [14-15] while the White Dorper is regarded as a breed of Tropical origin that was developed in arid subtropical South Africa for meat purpose in the 1940s. The breed has just been introduced in Hungary in 2008 [16]. This study aimed at understanding the dynamics of the relative gene expression of *IL-10* during the two seasons, in the two breeds, and among the sexes, with particular emphasis on heat stress conditions and the further goal of expounding the adaptability of the two breeds to heat stress situations.

2 Materials and Methods

2.1. Sample Collection and Location

Four Hungarian Indigenous Tsigai sheep and 4 White Dorper sheep were utilized in this study as experimental animals. Of them, 2 are ewes, and 2 are rams for each breed. The animals were housed in conventional deep litter type housing with several pen separation devices and implement that allow adequate airflow and access to the immediate environment during the hot season and enclosure during the wet and cold seasons. Under the regular farm feeding regimen, the animals were provided clean drinking water. All animals that were part of the study were of similar age (2 to 3 years old), body weight (ewes: 45 to 55 kg; rams: 65 to 75 kg), and in optimal health, exhibiting no physical or anatomical abnormalities.

About 5 ml whole blood sample was obtained from the jugular vein of the same animals in RNAprotect Animal Blood Tubes (QIAGEN, Hilden, Germany) and preserved at -70°C until further analysis.

The animals used in this study were kept in the Kismacs Experimental Station of Animal Husbandry of the University of Debrecen, located at 4002 Debrecen, Kismacs Tanya 4, Hungary. The elevation is 127 m above sea level and site coordinates are 47.58° N and 21.58° E.

2.2. Climatological Data

Data on the climatic condition during the sampling day, which include temperature and relative humidity, were recorded from 12:00 to 13:00 in the afternoon and obtained from the Hungarian Meteorological Service station in the vicinity. To calculate the THI for each sampling day, the equation by Mader et al. [17] was used.

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

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

The severity of heat stress in livestock is typically rated using the THI with ranges categorized as follows: no stress (≤ 67), mild (68–74), moderate (75–78), severe (79–83), and extreme (≥ 84) [18].

Additionally, in order to provide a broader and more objective overview of the climatic cycle and changes in meteorological variables as the seasons change in the area and to give background reference to the climatological data on sampling days, the 30-month climatological records (August 2018 to January 2021) [19] measured by the adjacent government meteorological station, were obtained from the Hungarian Meteorological Service.

2.3. Quantification of relative gene expression levels using RT-qPCR

Following the manufacturer's instructions, total RNA was extracted from sheep blood using the Rneasy Protect Animal Blood Kit (QIAGEN, Hilden, Germany). Using a NanoDrop ND-1000 Spectrophotometer, RNA quality and quantity were assessed (Thermo Fisher Scientific, Waltham, MA, USA). Total RNA of about 100 ng was reverse transcribed into cDNA with specific primers (Table 1) by the qPCRBIO cDNA Synthesis Kit (PCR Biosystems, London, United Kingdom). Forward and reverse primers (Table 1) were created with Primer Express v3.0.1 software (Applied Biosystems, Foster City, CA, USA) and confirmed for target Identity with Primer Blast from the National Center for Biotechnology Information (NCBI) (Ye et al., 2012). A 7300 Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) was used to conduct quantitative real-time PCR (qPCR). The 10 ll reactions consisted of 5 ll PowerUpTM SYBR! Green Master Mix (Applied Biosystems, Foster City, CA, USA), 0.6 ll each of 10 lM forward and reverse primers, 1.3 ml dH2O (MilliporeSigma, Burlington, MA, USA), and 2.5 ml cDNA (2 ng/ml). For relative expression studies, one housekeeping gene (glyceraldehyde 3-phosphate dehydrogenase (GAPDH)) was amplified alongside the target genes (*IL-10*). Quantitative PCR was done in triplicate for each sample.

Table 1. Detail of primers for qPCR analysis of the target genes

Target gene	Gen Bank accession	Primers	Fragment size (bp)
IL-10	NC_056065	F: TGATGCCACAGGCTGAGAAC R: CAGAAAACGATGACAGCGCC	110
GAPDH	NC_040254.1	F: CTGGCCAAGGTCATCCAT R: ACAGTCTTCTGGGTGGCAGT	86

2.4. Data and Statistical Analysis

The relative expression of the *IL-10* gene was determined by calculating the values using the equation proposed by Pfaffl (2001). The method compared the expression of *IL-10* gene with the reference gene GAPDH, with their amplification efficiencies both

corrected for differences. In seasonal comparison, the values for winter were used as the calibrator, while for sex differences, the rams' values were used as the calibrator.

$$Gene\ expression\ ratio = \frac{(E_{GOI})^{\Delta Ct\ GOI}}{(E_{HKG})^{\Delta Ct\ HKG}}$$

: EGOI- corrected efficiency of the gene of interest (GOI); EHKG- corrected efficiency of the housekeeping gene (HKG); ΔCt GOI- delta cycle threshold value of the GOI; ΔCt HKG- delta cycle threshold value of the HKG

Gene expression ratios means, standard deviation, and standard error were presented and graphically compared between the values for the summer and winter seasons and in-season comparison between rams and ewes.
Meteorological and gene expression data were downloaded and processed using Microsoft Excel (Microsoft Corporation, Redmond, WA), including tabular and graphical presentations [22-23].
For analysis, the differences of *IL-10* gene expression between seasons (summer and winter), between the breeds, and sexes (rams and ewes) in every season were determined through Student t-test [24] using the average cycle threshold (CT) values for the gene of interest. A difference with $P<0.05$ value was considered statistically significant. Data analysis was carried out using R program for Windows [25].

3 Results

3.1. Climatological variables

The 30-month climatological records, which span from 2018 to 2021, show the seasonal variations of meteorological variables such as temperature, relative humidity, and THI surrounding the study's sample collection period. Environmental temperature and air relative humidity were factors that affected THI. The highest THI values were observed during the summer months of June, July, and August, which correspond to high-temperature levels. Conversely, relative humidity falls during periods of high temperature, while it is high during low-temperature periods such as the winter season (Figure 1).

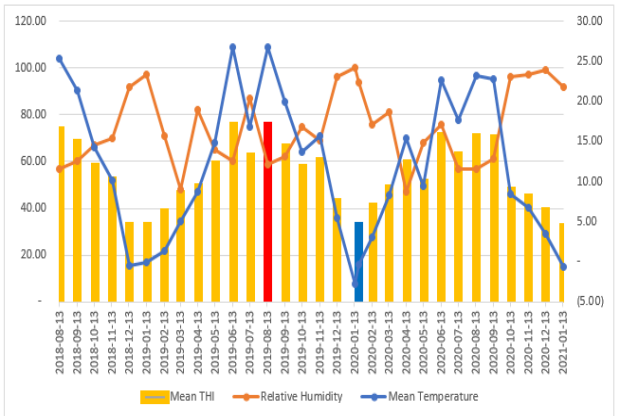

Figure 1. 30-month climatological record in the farm location. The mean THI values during sample collection days were highlighted in red (summer) and blue (winter) bars.

The data on the climatic condition during the collection of samples in summer and winter are presented in Table 2. The summer data collection day was characteristically hot and dry, with temperature recorded at 32.80°C and relative humidity at 34.50%. In contrast, the winter data collection day was cold and humid, with temperature recorded at -3.33 °C and relative humidity at 99.70%. The calculated THI during summer collection day was 79.0, indicating severe heat stress, while the THI during winter was 26.1, meaning there was no heat stress in the animals. Figure 2 shows the heat stress intensities that correspond with the THI values during the sample collection and entire study period.

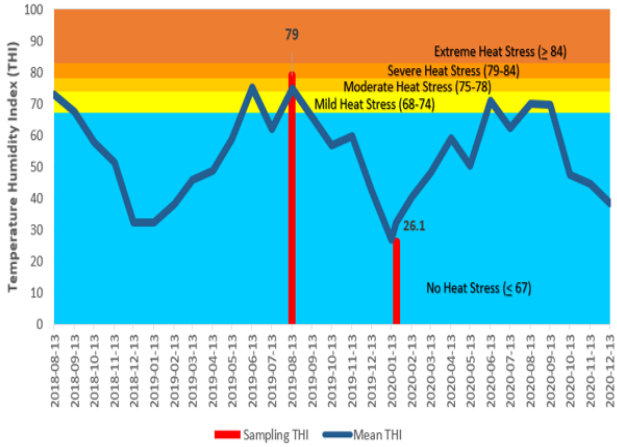

Figure 2. Heat stress levels according to THI values in the 30-month period with the THI values during the hours of sample collection, highlighted in red bars, indicating the levels of heat stress according to Lewis Baida et al. [18].

For most of the year, THI levels were below the heat stress threshold of 65. High THI values were recorded during the summer season, indicating heat stress conditions. Mean THI levels during the summer season show moderate heat stress, but there were times during the day that were marked with elevated THI values, and thus characterized heat stress to be severe. The hour of sample collection for summer shows that the animals were subjected to severe heat stress condition, with THI values at 79.0, several points higher than the mean THI during that day. On the contrary, the absence of heat stress was established during the winter sample collection with a THI of 26.1.

3.2. Expression of *IL-10* gene

Following qPCR analysis to determine the gene expression levels of *IL-10* in the experimental animals, relative expression was calculated using the Pfaffl method where the winter season values were used as calibrator. For all animals, mean gene expression ratio during the summer season was 16.82 with a standard deviation of 20.85 (Table 3). In contrast, the winter mean gene expression ratio was 1.18 with a standard deviation of 0.61. Relative gene expression ratios of *IL-10* in summer and winter seasons were presented in Figure 3 with winter season value as calibrator. A higher gene expression ratio of *IL-10* is found in the summer season as compared to the winter season ratio.

Table 2. Climatological data on the day of sample collection

Season	Sampling date	Sampling time	Temperature (◦C)	Relative humidity (%)	THI	Heat Stress Rate
Summer	13/ 08/ 2019	12.00-13.00	32.80	34.50	79.0	Severe
Winter	22/01/ 2020	12.00-13.30	-3.33	99.70	26.1	No Stress

Table 3. Relative Gene Expression of *IL-10* in Hungarian Indigenous Tsigai and Dorper Sheep during summer and winter seasons

Relative gene expression (Mean ± SD)		
Breed	**Summer**	**Winter**
Overall	16.82 ± 20.85	1.18 ± 0.61
Hungarian Indigenous Tsigai	12.3 ± 15.25	16.2 ± 18.53
White Dorper	1.1- ± 0.56	1.04 ± 0.35

SD: Standard Deviation

Breed-wise, the Hungarian Indigenous Tsigai sheep has an *IL-10* gene expression ratio mean of 12.3 and a standard deviation of 15.25 during summer. The White Dorper, on the other hand, has a mean of 16.2 and a standard deviation of 18.53 during summer. Here, the White Dorper showed a numerically higher ratio of gene expression during the summer seasons compared to the Hungarian Indigenous Tsigai.

The gene expression ratio of all the animals and the two breeds during the summer and winter are graphically presented and compared in Figure 3.

The average CT-values were used to statistically analyze differences among the gene expression in 2 seasons (Table 4), between the two breeds, and among sexes. In all animals, expression levels of *IL-10* were significantly higher (P=0.00019) during the summer season than those samples collected during the Winter season. Within-breed comparison of summer and winter CT-values showed significantly higher *IL-10* expression during the summer season in both breeds (Hungarian Indigenous Tsigai P= 0.015 & White Dorper P= 0.004). Between the two breeds, Hungarian Indigenous Tsigai and White Dorper, there is no significant difference in the CT-values during the summer and winter seasons. Similarly, no significant difference between ewes and rams was found during the two seasons in terms of relative gene expression ratio and CT-values (Table 5).

Table 4. Mean and standard deviation of CT-value of *IL-10* of Hungarian Indigenous Tsigai and White Dorper in summer and winter seasons.

CT-value (Mean ± SD)				
Season	**Hungarian Indigenous Tsigai**	**White Dorper**	**Overall**	**P-value**
Summer	32.87 ± 1.84[a]	32.62 ± 2.27[a]	32.74 ± 1.92[a]	*0.87*
Winter	29.37 ± 1.64[b]	28.09 ± 0.54[b]	28.86 ± 1.4[b]	*0.12*
Overall	31.25 ± 2.29	30.35 ± 3.20	30.80 ± 2.75	
P-value	*0.015*	*0.004*	*0.00019*	

SD: Standard Deviation; Note: The different superscripts show significantly different values (P < 0.05).

Table 5. Relative gene expression ratio and CT-value of *IL-10* of Ewe and Ram Hungarian Tsigai and White Dorper Sheep in summer and winter seasons.

	Hungarian Indigenous Tsigai			White Dorper		
Season	**Group**	**Relative Gene Expression Mean**	**CT-value Mean**	**Group**	**Relative Gene Expression Mean**	**CT-value Mean**
Summer	Ewe	0.01	34.205	Ewe	177.61	30.765
	Ram	0.06	31.53	Ram	1.05	34.475
Winter	Ewe	0.43	30.885	Ewe	0.6	30.885
	Ram	0.01	28.36	Ram	1.01	28.235

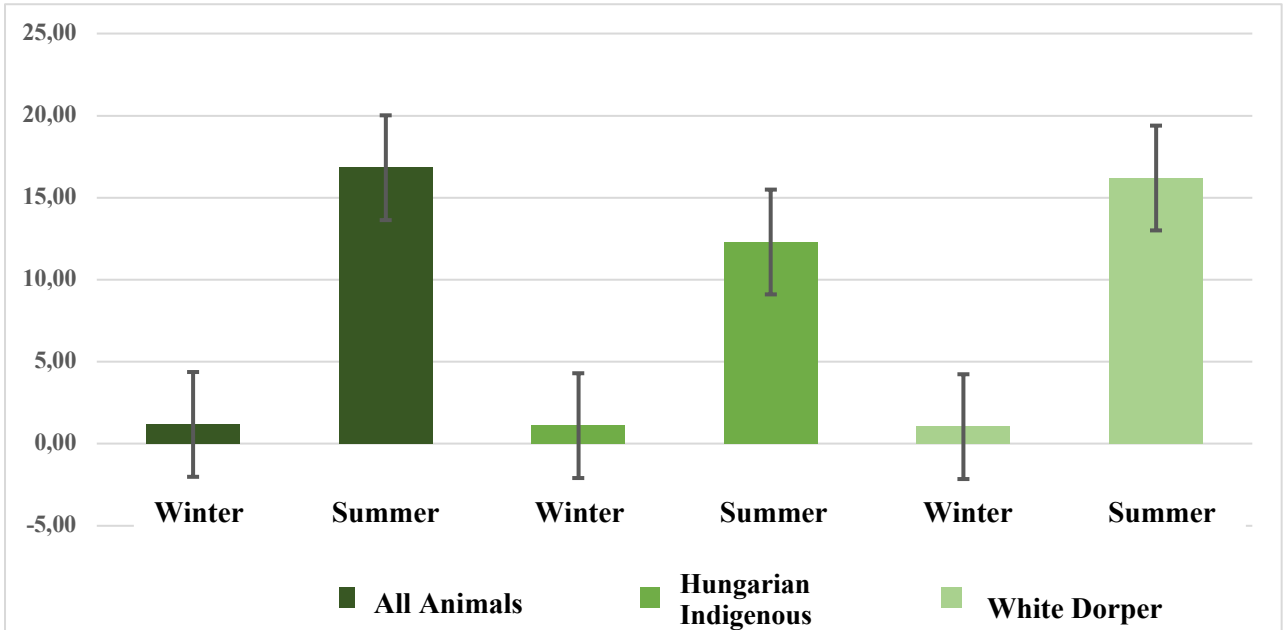

Figure 3. Bar graph of relative gene expression of *IL-10* in each season of the year with the winter season as the calibrator. The X-axis is the relative gene expression in the two seasons. The Y-axis is gene expression for all animals, and the two breeds studied. Different colors represent different breed.

4 Discussion

Maintaining thermoneutral conditions is one of the important requirements in ensuring the welfare and optimal production and reproduction performance of animals [26].

THI is considered as one of the most reliable measures and sensitive indicators of heat stress in animals, which integrates temperature and relative humidity parameters [27-28]. In this study, during the summer sample collection, THI was calculated to be high at 79.0, which indicates a state of severe heat stress. High temperature and low humidity are typical of summer seasons in the region. The average annual temperature in the immediate area was 11.66 °C for the period of 2011 to 2020 [29]. The average temperature recorded on the day of collection was 26.70 °C, a 176% deviation from the annual average. Factoring the daily maximum temperature and the relative humidity during the summer months of June to August [29], THI ranges from 69.67 to 88.80 for sheep, which indicates mild to extreme heat stress.

Consistent with the climatological data gathered and determined in this study, elevated THI in sheep during the summer season and hot periods that established a heat stress condition was also reported in Small tail Han sheep [30], Indian sheep [31], and Omani and Australian Merino sheep [32] where the animals exhibited increased respiratory rate, pulse rate, and rectal temperatures. Such signs are distinctive physiological indicators of heat stress response in animals [33-34].

High-THI conditions also influence metabolic and hematological parameters in sheep. Cortisol and biochemical parameters creatinine, zinc, and phosphorus were elevated during heat stress conditions and are highly correlated to THI levels in Suffolk sheep [35]. Sheep blood parameters chlorides, sodium, phosphorus, total protein, tetraiodothyronine, cholesterol, triglyceride, creatinine, cortisol, and glucose were likewise reported to be significantly increased by heat stress conditions in the study of Li et al. [30]. In the same way, values for red blood cell (RBC), hemoglobin (Hb%), and packed cell volume (PCV%) were significantly elevated in indigenous sheep subjected to heat stress [36].

The negative effects of heat stress on general welfare, comfort, and health could result in diminished immune function and increased incidence of diseases [37-38].

Primarily, the response of the immune system to abiotic stressors, such as heat stress, begins with the stimulation of primary and secondary lymphoid and myeloid organs that catalyze the synthesis and release of immune cells and agents [5]. Studies show that in acute heat stress (brief and intense), immune activation happens, while chronic heat stress causes immune suppression [39]. Elevated cortisol levels, as a result of the activation of the hypothalamic-pituitary axis (HPA), alter various immune function elements that may increase the animal's vulnerability to diseases [40]. This effect is demonstrated in the induction of inflammation in acute heat stress and the suppression of release and synthesis of inflammatory cytokines and the consequent changes to the dynamics of balance on the levels of other cytokines, immune cells, and substances in chronic situations [41-42].

Cytokines are key mediators of the immune system's response to stressors and pathogens. Proinflammatory cytokines work to activate the innate immunity for the body's early response against infectious agents and support the activities of the cell-mediated immune response of the adaptive immunity to synergistically combat the pathogen of concern. However, specific cytokines modulate the previous cytokines to suppress the immune response and shift the modality of the immune system into adaptive immunity. The most powerful and significant immunomodulatory cytokine of this effect is *IL-10* [43].

In the present study, overall *IL-10* gene expression in Hungarian Indigenous Tsigai and White Dorper sheep were shown to be significantly higher (P=0.015) in animals under severe heat stress (summer) compared to those that experienced no heat stress condition (winter). This result is in accordance with the findings of Caroprese et al. [44], who demonstrated high *IL-10* expression in sheep experiencing hyperthermia. Similarly, in cattle, *IL-10* levels were increased in heat-stressed Holstein cows [10, 45], Italian Friesian cows [46], and Sahiwal cows [11].

The expression of *IL-10* in heat stress situations has various physiological and immunological indications that help the animal adapt and restore equilibrium. While it suppresses the proinflammatory response, it leads the immune regime from TH1 to TH2 that activates the adaptive immunity [47-48]. Sustained *IL-10* levels during heat stress and the extended period after the heat stress situation and strenuous activities have effectively controlled immune response to stress [49].

Ripley [50] has reported that *IL-10* activates heat shock protein 90 (HSP90). Heat shock proteins are molecular chaperones that are another important factor for protecting cells from damage, such as in heat stress situations and in antigen presentation to improve both innate and adaptive immunity [51]. *IL-10* expression by the experimental animals in this study suggests that there could also be accompanying HSP expression, which also aids in the immune response and endogenous protection against heat stress.

Furthermore, *IL-10*, in line with its anti-inflammatory actions, has also been associated with the inhibition of cyclooxygenase (COX) and, consequently, prostaglandin E2 (PGE2), the principal downstream mediators of fever that are inducted by inflammatory cells [52]. This would mean that the expression of *IL-10* during heat-stress situations is thermo-modulatory and helps reduce heat load as it eliminates endogenous heat production by fever pathways.

Though all the sheep in this study show significantly higher expression of the *IL-10* gene during the summer season, the inter-breed comparison reveals a numerically higher gene expression ratio among the White Dorper (16.2) against the Hungarian Indigenous Tsigai (12.30). Statistical analysis of the CT-values, however, yielded no significant difference between the two breeds on the same subject. The two breeds, despite having different geographical origins, manifest comparable thermo-tolerance evident in the *IL-10* mRNA expression that bears no significant difference.

The Hungarian Indigenous Tsigai Sheep, being a temperate indigenous breed that thrived in the continental climate for centuries [15, 53], elucidated heat stress response in the distinctively harsh and hot summer environment of the region.

In a similar manner, the White Dorper sheep that is considered tropical in origin, maintained its heat stress responsive characteristic as it experienced the hot summer season in the study location where it was introduced a few years ago. Despite being present in Hungary for not a long time, the breed's tropical characteristics made acclimatization manageable for it to thrive during the hot summer months in the area.

Even though *IL-10* is a prominent immunological biomarker in heat stress-related studies in domestic animals, comparative studies of its mRNA expression across breeds, particularly between tropical and temperate breeds, are very scarce, especially on sheep.

In contrast to the findings of the present study, various related studies reported differences in *IL-10* gene expression among different breeds in domestic animals, such as cattle, during heat stress. Previous studies showed significantly higher *IL-10* gene expression in Sahiwal cows [11] and Karan Fries cows [54] compared to other breeds that include temperate ones. Citing other related thermotolerance immune biomarkers such as heat shock proteins (HSPs), Sahiwal, a tropical cattle breed, also exhibited higher mRNA expression of HSP90 [55] and HSP70 [56] compared to the temperate and tropical cross, Frieswal. Following this trend, the Caribbean sheep breed Pelibuey was found to be less susceptible to heat stress and with increased HSP70 concentration compared to Suffolk [57].

A comparative study in pigs revealed better cell viability in the tropical Creole pigs than the temperate Large White pigs, with a significant increase in the expression of HSPs. Interestingly, however, there was no difference in mRNA expression of HSP70.2 and HSP90 [58] in agreement with the findings of the current study.

The increase in *IL-10* concentration can result in the upregulation of HSPs gene expression in peripheral blood mononuclear cells, consequently leading to increased HSP concentration in circulation [50].

The equivalent heat tolerance in the Hungarian Indigenous Tsigai to the tropical breed White Dorper indicated by its *IL-10* gene expression in this study can be attributed to its indigeneity in the area that is shown in its hardiness and suitability to local environment. Its exposure to the local agro-climatic conditions across generations through centuries helped it adapt to the harsh summer months in the region. Its success in variable climatic conditions is proven by its wide distribution in the Central-Eastern European and Balkan regions being integrally used in sheep breeding programs confirmed by relatively low to moderate levels genetic variation among country populations [53, 59]. The tenacity of indigenous breeds in extreme climatic conditions can be ascribed to their physiological and genetic adaptations that have arisen over generations of slow modification and adaptation to environmental challenges [60, 28].

On another note, the excellent thermotolerant character of the White Dorper sheep, according to the parameters of this study, suggests its suitability for the harsh summer heat stress conditions in the area, despite its relatively short period of habitation. Tropical breeds can thrive comfortably in temperatures as high as 38 °C and produce adequately by virtue of thermoregulatory processes [61]. This characteristic was exhibited by Malpura ewes from the Indian semi-arid regions [62] and Santa Ines and Morada Nova sheep in Brazil [63]. Performance-wise, Dorper raised in the same area under Hungarian rearing conditions proved to have a good production and reproduction performance as reported in a study by Budai et al. [16].

In a pilot study evaluating reproductive performance and health indicators in the same area in Hungary, genotype differences were found in incidences of clinical mastitis (higher in Tsigai), lameness (higher in Dorper), and litter size (lower in White Dorper), while no differences were observed in conception and lamb survival rates [64]. It provides an overview of the variability of immune status among the breeds of interest, which merits the conduct of further investigation as to how heat stress could affect those incidences and the role of *IL-10* in such health challenges and reproductive performance.
Finally, in contrast with the findings of Li et al. [30], that reported better high-temperature tolerance in ewes over rams, the present study finds no sexual differences. The absence of sexual differences in the mRNA expression of *IL-10* in this study implies that thermotolerance and heat stress immune responses in both breeds are not influenced by sexual factors.

5 Conclusion

Overall, the study has demonstrated higher relative gene expression of the IL-10 gene during the summer season in both the Hungarian Indigenous Tsigai and White Dorper when heat the animals experienced heat stress conditions in contrast with the winter season. Implicated as a molecular biomarker for heat stress response, the significant relative gene expression of *IL-10* indicates immune modulation and shift to adaptive immune response. There has been no significant difference in the *IL-10* gene expression between the two breeds. While sexual factors do not affect *IL-10* gene expression, its expression can potentially enhance physiological adaptive mechanisms to avoid cellular damage during heat stress conditions in both breeds, regardless of sex.

Owing to their origins, the comparable heat stress response through the expression of the IL-10 gene proves that both breeds, continental and tropical in origin, are adaptive to the region's environmental conditions and could be utilized in breeding programs to improve the immunity, resiliency, and production performance of sheep, even in variable climatic conditions.

Further study on the links of genetic adaptability, physiological reactions, immune response, breeding programs, animal health, and productivity must be conducted to unravel complex mechanisms of homeostasis and animal performance in the face of heat stress, particularly involving *IL-10* and related biomarkers.

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