

HSP90AB1 (SNP-4338T>C) gene polymorphism associated with thermo-tolerance in some cattle breeds in Türkiye*

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ABSTRACT

Global warming is becoming a greater threat for the agricultural sector, while molecular genetics studies still hold new opportunities, to not only detect heat-tolerant animals, but also to allow for increasing the frequencies of desired genotypes in a certain population. In this study, *HSP90AB1* gene associated with heat tolerance was investigated in four cattle breeds known as Zavot (ZAV), Sout Anatolian Yellow (SAY), South Anatolian Red (SAR), and Brown Swiss (BS) reared in Türkiye via Allele-Specific Polymerase Chain Reaction (AS-PCR). 4338T>C mutation of the *HSP90AB1* gene yielded a total of three genotypes (CC, CT, and TT) across all cattle breeds in which C allele frequency ranged from 0.34 (SAY) to 0.73 (BS), while T allele frequency varied between 0.27 (BS) and 0.66 (SAY). In BS, CC was the genotype with the highest frequency (0.50), whereas the frequency of CC was lower than CT and TT in the Anatolian breed (ZAV, SAR, and SAY). Similarly, the frequency of TT was higher in native Anatolian breeds than BS (0.05). All the populations studied were in Hardy-Weinberg Equilibrium (HWE) in terms of the *HSP90AB1* gene. This study confirmed that the *HSP90AB1* gene was polymorphic in four cattle breeds reared in Türkiye. This polymorphism has the potential to allow for improving heat tolerance to maintain animal production in the future via suitable selection studies. Therefore, this polymorphism should be conserved in Anatolian cattle breeds, while other genes related to different environmental stressors may be monitored by further studies.

1. Introduction

During the last century, numerous developments, such as an enormous increase in the production level of agricultural and industrial sectors, improvement in global transportation networks as well as a rapid increase in the human population, have negatively affected the environment which can not be easily recovered (Bayram et al. 2023). Global warming-based problems are the main concern among these negative effects. It is estimated that temperature has increased by approximately 1°C from the beginning of the 20th century, while it is reported that this increase has reached up to nearly 2°C per decade after 1975. The Intergovernmental Panel on Climate Change (IPCC) has documented that the increase in temperature is expected to be 2-3°C higher at the end of the 21st century (Lu et al. 2020; Malhi et al. 2021; UNEP 2022). As highlighted by Demir et al. (2022a), climate change is considered to be a major threat to the sustainability of animal breeding systems across the globe since increased temperature not only negatively affects animal welfare but also leads to reproduction problems and a decrease in economically important yields (Demir et al. 2021a).

In this context, native animal breeds, which are believed to have developed adaptation against numerous climatic conditions, have gained more importance in maintaining animal production under environmental factors which are likely to change in the near future. Official data indicates that approximately 17 million cattle are reared in Türkiye of which 49.2% and 7.3% are represented by cosmopolitan and native breeds, respectively

(TUIK 2022). Holstein Friesian, BS, Simmental, and Jersey are the most preferred among the cosmopolitan breeds, while ZAV, SAR, SAY, Anatolian Black, East Anatolian Red (EAR), and Turkish Grey Steppe (TGS) are the native Anatolian cattle breeds (Demir et al. 2021b; Demir et al. 2022b). Of these native breeds, SAR is reared in the southern part of Anatolia including Mersin, Adana, Gaziantep, and Şanlıurfa provinces for milk and beef production. SAY is also reared in the southern part of Anatolia for dual purpose, whereas it is distinct from other native cattle breeds in terms of its coat colour and climbing ability to mountainous areas. ZAV is raised by smallholder farmers in the eastern part of Anatolia including Kars and Ardahan provinces, particularly for milk production. Native Turkish cattle breeds are of lower yields in terms of milk and beef compared to cosmopolitan breeds, while they have developed excellent adaptation against local diseases and climatic conditions of Anatolia (GDARP 2009; Demir et al. 2021b).

Possessed by native breeds, adaptive traits, which have been shaped by numerous factors from domestication till the present, and their underlying reasons have always been considered interesting study areas for scientists working on molecular genetics. Indeed, many previous molecular studies have revealed numerous genes such as *KRT77*, *MYO1A*, *BoLA-DRB3* which are directly associated with environmental stressors (Duangjinda et al. 2013; Jia et al. 2019; Zhang et al. 2023) When it comes to heat stress and tolerance to higher temperatures in livestock species,

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Heat Shock Proteins (HSPs) and variations in their related genomic regions have been mostly investigated (El-Zarei et al. 2019; Irvboje et al. 2020; Rawash et al. 2022; Guzmán et al. 2023). HSPs showing differences in terms of their molecular weight and biological functions are molecular chaperons activated during heat and related stress conditions. During cellular damage, HSPs are released into the blood in order to protect living cells from toxic effects and heat-related stress factors. Based on their molecular weights, HSPs are categorized into five groups such as HSP100, HSP90, HSP70, HSP60, and small HSPs whose molecular weights are estimated at 17-30 kDa (Yer 2017; Çıldır and Özmen 2019; Şenel et al. 2019). HSP90AB1 gene has been mapped to bovine chromosome 23 (with 724 amino acids) and consist of 12 exons (Prastowo et al. 2021; Hariyono and Prihandini 2022).

Several studies have confirmed that variations in HSP gene regions were directly associated with thermal tolerance in different cattle (Kumar et al. 2022), sheep (Sheraz et al. 2023), goat (Mohanarao et al. 2014), and chicken (Sheraiba et al. 2019) breeds. To illustrate, Sajjanar et al. (2015) investigated the effects of genetic variations in the *HSP90AB1* (SNP g.4338T>C) gene on heat tolerance and milk production in Sahiwal and Frieswal cattle breeds via Allele-Specific Polymerase Chain Reaction (AS-PCR). A total of three genotypes called CC, CT, and TT were declared and animals with TT genotype were reported to be more advantageous in terms of heat tolerance and respiration rate based on association analysis (Sajjanar et al. 2015). Moreover, the authors highlighted that *HSP90AB1* (SNP g.4338T>C) polymorphism could be utilized as a molecular marker to improve heat tolerance in cattle breeds. On the other hand, few studies are available in the literature attempting to investigate HSP polymorphisms in native Turkish livestock species (Öner et al. 2017; Atalay and Kök 2023; Yurdagül et al. 2023). Hence, this study aims to screen four cattle breeds (SAY, SAR, ZAV, and BS) reared in Türkiye in terms of *HSP90AB1* (SNP g.4338T>C) polymorphism which was previously reported to be associated with heat tolerance in cattle. In the case of observing the TT genotype, it is also aimed to evaluate the usefulness of this polymorphism in Marker Associated Selection (MAS) which may be taken into consideration for further management practices.

2. Material and Methods

2.1. Ethic statement

This research was approved by the Local Ethics Committee of Animal Experiments of the Eskişehir Osmangazi University (Protocol No: HAYDEK-970/2023).

2.2. Sample collection and DNA extraction

A total of 117 animals (from both sexes) belonging to ZAV (n= 30), SAY (n= 34), SAR (n= 31), and BS (n= 22) were sampled from different representative herds (at least three herds per breed) to prevent possible kinship. Blood samples of ZAV, SAR, and SAY were collected from herds located in Kars, Şanlıurfa, and Adana provinces, respectively, while blood samples of the BS breed were obtained from different herds reared in Antalya province (Figure 1). The salting-out protocol described by Miller et al. (1988) was used to total DNA extraction from blood.

2.3. Determination of *HSP90AB1* (SNP-4338T>C) gene polymorphism

In this study, the AS-PCR protocol described by Sajjanar et al. (2015) was used to amplify a 562 base pair length of the *HSP90AB1* region to screen 4338T>C SNP in four cattle breeds. As recommended two specific primers were used to amplify the forward strand for C (CTGGAGTCACACTGAGGAAC) and G (CTGGAGTCACACTGAGGAAT) alleles, while a common primer (TGTTGGAGATCGTCACCTG) was used to amplify the reverse strand for both alleles. PCR reaction of 30 µl (4 µl 10X PCR buffer, 10 mM MgCl₂, 2.5 mM dNTPs, 10 pmol each primer, 50 ngP µl template DNA, 2.5 U Taq DNA polymerase, and 12.9 µl ddH₂O) was subjected to thermal cyclers as follows: initial denaturation at 95°C for 5 min, followed by 30 cycles of denaturation at 95°C for 45 s, annealing at 63°C for 45 s, and extension at 72°C for 45 s, while final extension was optimized at 72°C for 5 min. Amplified PCR fragments were separated on 1.5% agarose gel electrophoresis in which individuals with two amplifications were genotyped as CT, while others were genotyped as TT or CC based on the presence of the amplified allele.



Figure 1. Geographical representation of sampling strategy.

2.4. Statistical analysis

Popgene v.1.32 (Yeh et al. 1997) was used both to calculate allele and genotype frequencies and to test Hardy-Weinberg Equilibrium (HWE) across four cattle breeds in terms of *HSP90AB1* (SNP g.4338T>C) polymorphism. Minitab software was also utilized to compare genotype frequencies within (two dependent proportions) and between (two independent proportions) populations via z test.

3. Results and Discussion

In this study, the *HSP90AB1* gene region turned out to be polymorphic in terms of 4338T>C mutation in four cattle breeds. The allele and genotype frequencies across the cattle breeds studied are summarized in Table 1, while a representative agarose gel electrophoresis including three genotypes (CC, CT, and TT) for nine animals belonging to SAR is given in Figure 2.

C allele frequency ranged from 0.34 (SAY) to 0.73 (BS), while T allele frequency varied between 0.27 (BS) and 0.66 (SAY) across the cattle breeds studied (Table 1). The lowest (0.13) and highest (0.50) CC genotype frequencies were observed in ZAV and BS breeds, respectively. CT genotype frequency ranged from 0.35 in SAR to 0.60 in ZAV, while TT allele frequency varied between 0.05 (BS) and 0.47 (SAR) (Table 1). Chi-square approach revealed that all cattle breeds were in HWE in terms of the *HSP90AB1* (4338T>C) gene region.

Sajjanar et al. (2015) investigated the associations between the *HSP90AB1* (4338T>C) gene variations and heat tolerance parameters (heat tolerance coefficient, average respiration rate, and average rectal temperature) and milk production in a total of 200 animals belonging to Sahiwal (n= 80) and Frieswal (n= 120). *HSP90AB1* (4338T>C) gene was shown to be polymorphic in both native Indian cattle breeds (Sahiwal and Frieswal). CC, CT, and TT genotype frequencies were reported as 0.20, 0.70, and 0.10, respectively for the Sahiwal breed, while these values were 0.05, 0.78, and 0.17, respectively for the Frieswal (Sajjanar et al.

2015). Compared to animals with CT and CC genotypes, a higher heat tolerance coefficient and lower average respiration rate and rectal temperature were reported in animals with TT genotypes in both cattle breeds ($P<0.01$). The authors have suggested that the TT genotype could be utilized to improve heat tolerance in cattle breeds, while other controlling mechanisms and related genes should not be neglected (Sajjanar et al. 2015). Another study conducted by Prastowo et al. (2021) revealed that CC, CT, and TT frequencies were 0.10, 0.50, and 0.40 in twenty animals belonging to Holstein Friesian reared in Indonesia in terms of the *HSP90AB1* (4338T>C) gene polymorphism.

TT genotype frequencies in ZAV (0.27), SAY (0.47), and SAR (0.35) breeds were higher than the values reported by Sajjanar et al. (2015) for Sahiwal (0.17) and Frieswal (0.10) breeds. On the other hand, a lower TT genotype frequency was detected for the BS breed (0.05) compared to both Anatolian and Indian native cattle breeds. The differences in the distribution of genotype frequencies among different cattle breeds may be explained by their genetic origins. Cattle breeds raised in Türkiye such as ZAV, SAY, SAR, and BS as well as Holstein Friesian investigated by Prastowo et al. (2021) are descendants of *Bos taurus*. On the other hand, Sahiwal originates from *Bos indicus*, while Frieswal has been developed via crossbreeding practices between *Bos taurus* and *Bos indicus*.

Normally, indicine cattle breeds are expected to be more heat-tolerant animals compared to taurine cattle breeds (Gaughan et al. 2010). Therefore, the frequency of the TT allele, which showed superior values in terms of heat tolerance as reported by Sajjanar et al. (2015), is expected to be higher than the values reported in taurine cattle breeds. However, both the results of the current study and the values reported by Prastowo et al. (2021) showed higher TT genotype frequency in different taurine cattle breeds. On the other hand, it is noteworthy that the number of animals used in the current study and Prastowo et al. (2021) was lower compared to the study conducted by Sajjanar et al. (2015).

Table 1. Allele and genotype frequencies and chi square values for the *HSP90AB1* (SNP-4338T>C) across four cattle breeds

| Breed | n | Allele frequency | | Genotype frequency | | | χ^2 |
|-------|----|------------------|------|-------------------------|-------------------------|--------------------------|--------------------|
| | | C | T | CC | CT | TT | |
| ZAV | 30 | 0.43 | 0.57 | 0.13 (4) ^{Bc} | 0.60 (18) ^{Aa} | 0.27 (8) ^{Bb} | 1.475 ^d |
| SAY | 34 | 0.34 | 0.66 | 0.15 (5) ^{Bc} | 0.38 (13) ^{Ab} | 0.47 (16) ^{Aa} | 0.724 ^d |
| SAR | 31 | 0.47 | 0.53 | 0.30 (9) ^{Ab} | 0.35 (11) ^{Ab} | 0.35 (11) ^{Aab} | 2.560 ^d |
| BS | 22 | 0.73 | 0.27 | 0.50 (11) ^{Aa} | 0.45 (10) ^{Ab} | 0.05 (1) ^{Bc} | 0.468 ^d |

Comparison of genotype rates within and between populations are given as lower- and upper-case letters, respectively ($P<0.05$). $\chi^2_{0.05,1}$: 3.84; d: Deviation from HWE is non-significant.

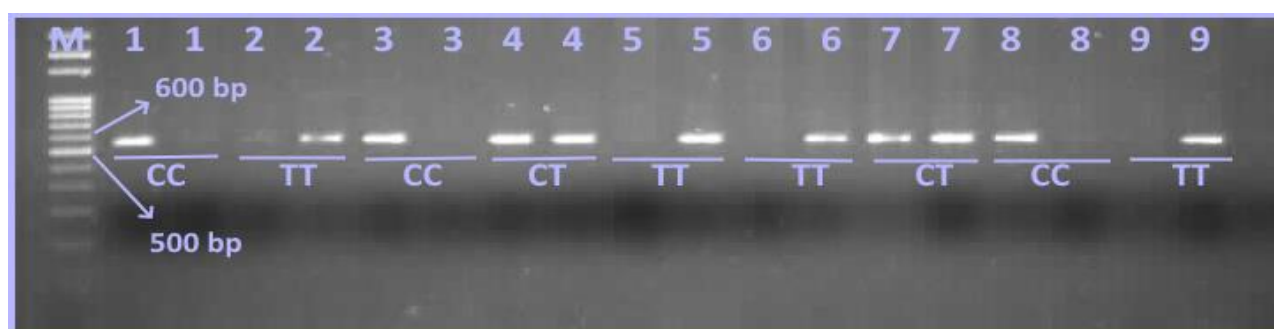


Figure 2. Image of 1.5% agarose gel electrophoresis for nine samples of SAR breed. M: Marker (100 bç- Thermo 100 bp; Cat.No: SM0241); 1, 3, and 8: animals with CC genotype; 4 and 7: animals with CT genotype; 2, 5, 6, and 9: animals with GG genotype.

Numerous studies are available in the literature indicating that native Anatolian cattle breeds are significantly tolerant to local diseases and environmental conditions compared to cosmopolitan cattle breeds (Bilgen et al. 2016; Karayel and Karšli, 2022; Çobanoğlu and Ardiçlı 2022). The results of this study are relevant to the literature because TT genotype frequency in three Anatolian cattle breeds (ZAV, SAY, and SAR) was significantly ($p < 0.05$) higher than the value detected in the BS breed (0.005). Among the Anatolian cattle breeds the highest TT genotype frequencies were observed in SAY (0.47) and SAR (0.35), respectively and differences between these breeds were statistically non-significant. However, differences in TT genotype between these breeds (SAR and SAY) and ZAV were statistically significant ($P < 0.05$). This finding is compatible with their geographic distributions. Indeed, SAR and SAY are mainly reared in the eastern Mediterranean and south-eastern Anatolia. These locations are of the highest temperature in Anatolia. Therefore, these breeds are thought to have developed an adaptation against higher temperatures over a long period of time.

4. Conclusion

Due to the fact that the trends in increasing temperature will continue globally in the future and threaten sustainable livestock breeding, not only thermo-tolerant animals should be detected but also their frequency should be increased via suitable management practices. In this study, the TT genotype of *HSP90AB1* (4338T>C) gene polymorphism, which was reported to be the tolerant genotype for heat stress, was detected at enough frequency in three Anatolian cattle breeds. The animals with TT genotype may be utilized in MAS programs to face increasing global warming in the future. On the other hand, other native Turkish cattle breeds need to be screened for heat-related genomic regions. Moreover, further studies may be conducted via higher-resolution molecular genotyping methods such as SNP arrays and next-generation sequencing platforms together with thermo-physiological parameters.

References

- Atalay S, K k S (2023) The Comparison of Polymorphisms in the Heat Shock Transcription Factor 1 Gene of Turkish Grey Cattle and Holstein Cattle. *Kafkas Universitesi Veteriner Fak ltesi Dergisi* 29(5): 429-435.
- Bayram A, Marvuglia A, Gutierrez TN, Weis J P, Conter G, Zimmer S (2023) Sustainable farming strategies for mixed crop-livestock farms in Luxembourg simulated with a hybrid agent-based and life-cycle assessment model. *Journal of Cleaner Production* 386: 135759.
- Bilgen N, Cinar Kul B, Offord V, Werling D, Ertugrul O (2016) Determination of genetic variations of Toll-like receptor (TLR) 2, 4, and 6 with next-generation sequencing in native cattle breeds of Anatolia and Holstein Friesian. *Diversity* 8(4): 23.
- Çıldır ÖŞ, Özmen Ö (2019) Sıcak stresi ve termotolerans: Heat stress and thermotolerance: Molecular studies in cattle. *Livestock Studies* 59 (Ek Sayı): 117-129.
- Çobanoğlu Ö, Ardiçlı S (2022) Genetic variation at the OLR1, ANXA9, MYF5, LTF, IGF1, LGB, CSN3, PIT1, MBL1, CACNA2D1, and ABCG2 loci in Turkish Grey Steppe, Anatolian Black, and EastAnatolian Red cattle. *Turkish Journal of Veterinary and Animal Sciences* 46(3): 494-504.
- Demir E, Bilginer U, Balcioglu MS, Karšli T (2021a) Direct and indirect contributions of molecular genetics to farm animal welfare: a review. *Animal Health Research Reviews* 22(2): 177-186.
- Demir E, Karšli T, Balcioglu MS (2021b) A comprehensive review on genetic diversity and phylogenetic relationships among native Turkish cattle breeds based on microsatellite markers. *Turkish Journal of Veterinary and Animal Sciences* 45(1): 1-10.
- Demir E, Ceccobelli S, Bilginer U, Pasquini M, Attard G, Karšli T (2022a) Conservation and selection of genes related to environmental adaptation in native small ruminant breeds: a review. *Ruminants* 2(2): 255-270.
- Demir E, Moravčíková N, Karšli T, Kasarda R (2022b) Future perspective of NGS data for evaluation of population genetic structure in Turkish cattle. *Acta fytotechnica et zootechnica* ISSN 1336-9245-25(2).
- Duangjinda M, Jindatajak, Y, Tipvong, W, Sriwarothai, J, Pattarajinda, V, Katawati S, Boonkum W (2013) Association of BoLA-DRB3 alleles with tick-borne disease tolerance in dairy cattle in a tropical environment. *Veterinary Parasitology* 196(3-4): 314-320.
- El-Zarei MF, Alosef AM, Alhaidary AA, Mousa EF, Okab AB, Samara E M, Abdoun KA (2019) Short-term heat shock proteins 70 and 90 mRNA expression profile and its relation to thermo-physiological parameters in goats exposed to heat stress. *International journal of biometeorology* 63: 459-465.
- Gaughan JB, Mader TL, Holt SM, Sullivan ML, Hahn GL (2010) Assessing the heat tolerance of 17 beef cattle genotypes. *International Journal of Biometeorology* 54: 617-627.
- GDARP (2009) Introduction catalogue of Turkish domestic animal genetic resources (in Turkish). <https://www.tarimorman.gov.tr/TAGEM/Belgeler/yayin/Katalog%20T%C3%BCrk%C3%A7.pdf>. Accessed 28 November, 2023.
- Guzmán LF, Martínez-Velázquez G, Villaseñor-González F, Vega-Murillo VE, Palacios-Fránquez JA, Ríos-Utrera Á, Montaño-Bermúdez M (2023) Expression of heat shock protein genes in Simmental cattle exposed to heat stress. doi: 10.5713/ab.22.0266.
- Hariyono DNH, Prihandini PW (2022) Association of selected gene polymorphisms with thermotolerance traits in cattle—A review. *Animal Bioscience* 35(11): 1635.
- Irivboje YI, Sanni MT, Fafiolu AO, Olowofeso O, Ikeobi CON (2020) Genetic polymorphisms in part of intron 7 and exon 8 of HSP90AA1 gene and its association with heat tolerance traits in two exotic layer chicken strains. *Tropical animal health and production* 52: 969-977.
- Jia P, Cai C, Qu K, Chen N, Jia Y, Hanif Q, Liu J, Zhang J, Chen H, Huang B, Lei, C (2019). Four novel SNPs of MYO1A gene associated with heat-tolerance in Chinese cattle. *Animals* 9(11): 964.
- Karayel F, Karšli T (2022) Genotypic structure of four cattle breeds raised in Turkey by loci related to several diseases. *Mediterranean Agricultural Sciences* 35(1): 39-45.
- Kumar R, Gupta ID, Verma A, Singh S, Kumari R, Verma N (2022) Genetic polymorphism in HSPB6 gene and their association with heat tolerance traits in Indian Karan Fries (Bos taurus x Bos indicus) cattle. *Animal Biotechnology* 33(7): 1416-1427.
- Lu H, Guan Y, He L, Adhikari H, Pellikka P, Heiskanen J, Maeda E (2020) Patch aggregation trends of the global climate landscape under future global warming scenario. *International Journal of Climatology* 40(5): 2674-2685.
- Malhi GS, Kaur M, Kaushik P (2021) Impact of climate change on agriculture and its mitigation strategies: A review. *Sustainability* 13(3): 1318.
- Miller S, Dykes D, Plesky HA (1988) Simple Salting out Procedure for Extracting DNA from Human Cells *Nucleic Acids Research* 16: 1215.
- Mohanarao GJ, Mukherjee A, Banerjee D, Gohain M, Dass, G, Brahma B, De S (2014) HSP70 family genes and HSP27 expression in response to heat and cold stress in vitro in peripheral blood mononuclear cells of goat (Capra hircus). *Small Ruminant Research* 116(2-3): 94-99.
- Öner Y, Keskin A, Üstüner H, Soysal D, Karakaş V (2017) Genetic diversity of the 3' and 5' untranslated regions of the HSP70. 1 gene

- between native Turkish and Holstein Friesian cattle breeds. South African Journal of Animal Science 47(4): 424-439.
- Prastowo S, Adzdzakiy MM, Vanessa R, Pambuko G, Susilowati A (2021) Polymorphism scanning of HSP90AB1 gene in local Friesian Holstein as molecular marker for heat stress resistance. In E3S Web of Conferences EDP Sciences 306, 05016.
- Rawash RAA, Sharaby MA, Hassan GEDA, Elkomy AE, Hafez EE, Hafsa SHA, Salem MM (2022) Expression profiling of HSP 70 and interleukins 2, 6 and 12 genes of Barki sheep during summer and winter seasons in two different locations. International Journal of Biometeorology 66(10): 2047-2053.
- Sajjanar B, Deb R, Singh U, Kumar S, Brahmane M, Nirmale A, Bal SK, Minhas P (2015) Identification of SNP in HSP90AB1 and its association with the relative thermotolerance and milk production traits in Indian dairy cattle. Animal Biotechnology 26(1): 45-50.
- Şenel A, Duman E, Türel ÖO (2019) Genetic adaptation mechanisms against changing climate conditions on farm animals. Gaziosmanpaşa Üniversitesi Tıp Fakültesi Dergisi 11(1): 1-23.
- Sheraiba N, Hemeda S, Mahboub H (2019) HSP70 and HSP90 β genes polymorphism and its association with thermotolerance in Fayoumi and Leghorn chicken breeds. Journal of Current Veterinary Research 1(2): 56-62.
- Sheraz M, ud Din F, Un Nabi MS, Rao S, Shafiq B, Murtaza G, Farooq M, Iqbal F (2023). Association of single nucleotide polymorphisms in heat stress protein 70 (HSP70) and 90 (HSP90) with the susceptibility of Pakistani sheep breeds to hemoparasitic infections. Animal Biotechnology 1-8.
- TUIK (2022) Statistical Tables, Number of Bovine Animals, <https://data.tuik.gov.tr/Kategori/GetKategori?p=tarim-111&dil=1>. Accessed: 28 November, 2023.
- UNEP (2022) United Nations Environment Programme, Emissions gap report, The closing window-Climate crisis calls for rapid transformation of societies, Nairobi.
- Yeh FC, Yang RC, Boyle TBJ, Ye ZH, Mao JX (1997) POPGENE, The user-friendly shareware for population genetic analysis. Molecular Biology and Biotechnology Centre, University of Alberta, Canada.
- Yer EN (2017) Genome-Wide survey of heat shock proteins (hsp) and expression analysis of hsp genes under abiotic stress conditions in some Populus taxons. PhD Thesis, Kastamonu Üniversitesi, Fen Bilimleri Enstitüsü, Kastamonu.
- Yurdagül KG, Sude A, Bilginer Ü, Karlı T, Demir E (2023) Genetic variations in HSP90AA1 gene region in Pırlak sheep breed. Hayvansal Üretim 64(1): 12-16.
- Zhang M, Wu D, Ahmed Z, Liu X, Chen J, Ma, J, Wang M, Liu J, Zhang J, Huang B, Lei C (2023) The genetic secrets of adaptation: decoding the significance of the 30-bp insertion in the KRT77 gene for Chinese cattle. Animal Biotechnology 1-8.