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# A Determination of the Change in Variance Components due to Heat Stress in Dairy Cattle Using a Random Regression Model

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#### ABSTRACT

The aim of this study is to evaluate changes in variance components for dairy cows under heat stress conditions using a random regression model. The daily milk yield and pedigree records used in the research were obtained from a dairy farm in Sanliurfa, Türkiye. The records were from Holstein dairy cows registered between 2017 and 2019 at the farm. A total of 690 lactations from 690 healthy dairy cows were used in the study and the total number of cow-days was 207,003. In order to evaluate heat stress on animals meteorological data were used and collected from a public weather station located 15.04 km away from the farm. In the study, variance components were separately estimated for the comfort period (CP) and the heat stress period (HSP) using a random regression test-day model and six-knot linear spline function was used. In the study, it was observed that heat stress resulted in an increase in additive genetic,

permanent environmental, and consequently, phenotypic variance. During the lactation period, the average heritability was determined to be  $0.13\pm0.007$  for CP, while it was found to be  $0.18\pm0.010$  for HSP. According to the findings obtained from the study, it was concluded that the time periods for selection should coincide with the peak milk yield under heat stress conditions, while for the period without heat stress, it should be around the  $120^{\text{th}}$  day of lactation. These results indicate that climatic factors such as temperature and humidity should be included in the models used for genetic parameter and breeding value estimation. Thus, it may be possible to identify dairy cattle that are genetically more tolerant to hot conditions. In this way, more successful outcomes can be achieved in selection studies.

Keywords: Dairy cattle, Genetic analyses, Temperature-humidity index, Eigenfuction, Weather station

# **1. Introduction**

It has been reported in many studies that heat stress negatively affects milk production and reproduction in dairy cattle (Bryant et al. 2007, Ravagnolo & Misztal 2002; Jordan 2003; Garcia-Ispierto et al. 2007; Polsky et al. 2017). Moreover, the adverse effects of global warming will only lead to more serious problems for dairy farmers.

According to Ravagnolo et al. (2000), since daily yields are impacted by weather conditions and reflect the effect of temperature and humidity, meteorological data obtained from public weather stations contain useful information for studies on heat stress in dairy cattle. This means that when weather conditions, such as temperature and humidity, prior to the test days are recorded, the effect of heat stress on animals can be predicted. Similarly, Freitas et al. (2006) reported that public weather data are reliable sources of information, as they have been found to be consistent with on-farm weather measurements. Misztal (1999) suggested a method for examining the genetic basis of heat stress in dairy cows, which involved utilizing performance records and publicly available weather data. In this method, unlike heat stress studies that rely on body temperature or respiration information (Gonzalez-Rivas et al 2018; Osei-Amponsah 2020), individual animal measurements are not required, allowing for the use of large datasets required for genetic evaluation. There is a significant genetic component to heat tolerance in firstlactation cows, particularly for milk, fat, and protein production, and the level of additive genetic variance at a high temperaturehumidity index (THI) was observed to be comparable to the additive variance observed under non-stressful conditions (Ravagnolo & Misztal 2000). In addition to this, Aguilar et al. (2009) reported a substantial increase in the additive genetic effects associated with heat stress and yield traits from the first to third parity. Furthermore, female calves of bulls that possessed high genetic merit for heat tolerance exhibited lower milk yields, higher milk solids contents, more robust body types, better udders, longer productive lives, and higher pregnancy rates in comparison to the female calves of bulls with low genetic merit for heat tolerance. The heat tolerance of dairy cattle can be impacted upon by intensive sire selection, especially in temperate climates. If there is a negative genetic correlation between production and heat tolerance, ongoing selection for production will lead to a gradual decline in heat tolerance (Ravagnolo & Misztal 2000).

Random regression models (RRM) are frequently employed for the analysis of longitudinal data in animal breeding (Schaeffer 2004). These models can incorporate various functions to capture the (co)variance structures across days in milk (DIM). One common approach is to use splines to model the (co)variances in test-day models (White et al. 1999; Druet et al. 2003; Silvestre et al. 2005; Bohmanova et al. 2008). According to Misztal (2006), the numerical properties of linear splines are advantageous, and they exhibit localized effects, making them a favourable choice for modelling (co)variance structures in longitudinal data. Moreover, their ease of interpretability adds to their utility for data analysis.

Sanliurfa province is situated in the Southeastern Anatolia Region of Türkiye and is recognized as one of the country's hottest provinces. The weather in this area is characterized by hot and dry conditions between July and October, with temperatures occasionally soaring to 46.8 °C. The region experiences an annual average of 459.3 mm of precipitation and a relative humidity of 51% (Anonymous 2023a). For this reason, milk production in Sanliurfa, especially in summer, is adversely affected due to heat stress. This raises the possibility that, in addition to milk yield losses, the impact of the important environmental factor of temperature and humidity could lead to errors in estimating breeding values.

In previous studies on this region and nearby provinces (Yazgan 2017; Demir & Yazgan 2023) milk yield losses due to heat stress were detected, but no study was conducted on the effect of heat stress on variance components. The aim of this study is to evaluate changes in variance components for dairy cows under heat stress conditions using a random regression test-day model.

# 2. Material and Methods

### 2.1. Data

The daily milk yield and pedigree records used in the research were obtained from a dairy farm in Sanliurfa, affiliated with The General Directorate of Agricultural Enterprises (TIGEM), a public institution. The farm is located at 36°48'46" N latitude and 39°51'57" E longitude, with the altitude of 408 meters. Records were from Holstein dairy cows registered between 2017 and 2019 at the farm. The cows were housed in an open free-stall barn system, provided with ad libitum access to feed and water, and milked twice daily using an automated milking system that recorded their milk yield. In the dataset used for the study, each lactation record belongs to a different animal. There are no multiple lactation records for the same animal in the dataset. In other words, the number of lactations and the number of animals in the dataset are equal to each other. All lactation records were restricted to those between 5 and 305 DIM and number of minimum daily records for a lactation were 299. A total of 690 lactations from healthy 690 dairy cows were used in the study. Among these lactations, 278, 130, 135, and 147 were the first, second, third, and fourth or higher lactations, respectively. In addition to this, the total number of cow-days was 207,003 (The number of daily milk yield records in total lactations). The milk production data and pedigree information are summarized in Tables 1 and 2.

			Milk yield (kg)		
OLP	N	n	$\overline{X} \pm S_{\overline{X}}$		
1	278	83 400	$17.65 \pm 0.015$		
2	130	39 004	$17.44 \pm 0.022$		
3	135	40 497	$17.01 \pm 0.024$		
$\geq 4$	147	44 102	$16.09 \pm 0.025$		
Total	690	207 003	$17.15 \pm 0.010$		

Table 1- Descriptive statistic of milk production data

OLP: Order of lactation parity (Each animal has only one lactation record), N: Number of lactations (at the same time, the number of animals), n: The number of daily milk yield records in lactations

Item	n
The total number of animals in the pedigree file	1316
Number of animals with milk yield records;	
Animals	690
Sire	80
Dams	634
Animals with unknown sire	9
Animals with unknown dam	-
Animals with unknown parents	-

The daily maximum, minimum, and average temperature and humidity data were collected from a public weather station in Sanliurfa, which is operated by the Turkish State Meteorological Service authorized by the Ministry of Environment, Urbanization, and Climate Change of the Republic of Türkiye. The location of the meteorological station is 36°50'26.2" N latitude and 40°01'50.5" E longitude, with an altitude of 363 meters. While the distance between the public weather station and

the farm was 15.04 km as a straight line (crow flies), the altitude difference between the farm and the public weather station was only 45 m.



Figure 1- Three-year (2017-2019) average of THI values

According to a previous study (Demir & Yazgan 2023), using the maximum temperature and minimum humidity in the THI formula better represents the stress conditions that animals are exposed to as a result of temperature and humidity in the hot-dry Southeastern Anatolia Region of Türkiye. Similarly, Ravagnolo et al. (2000) stated that the most crucial variables for measuring heat stress were the maximum daily air temperature and minimum daily humidity for calculation of THI. For this reason, in our study, the THI formula proposed by Ravagnolo et al. (2000) was used to determine heat stress, which includes maximum temperature and minimum humidity values (Eq. 1):

$$THI = (1.8t + 32) - (0.55 - 0.0055 \times rh)(1.8t - 26.8)$$
(1)

Where; t is temperature in degrees Celsius and rh is relative humidity, expressed as a percentage. For this, heat stress in dairy cattle begins at a THI value of 72, which is equivalent to 22 °C at 100% humidity, 25 °C at 50% humidity, or 28 °C at 20% humidity. Figure 1 shows calculated THI values for each day of the year (averaged over 3 years) for the present data set. Each daily milk yield record was assigned the daily THI values of the previous days and put together with the daily milk production data.

### 2.2. Models and statistical analyses

The following model was used to calculate the least squares means for milk yield according to THI values (Eq. 2):

$$Y_{ijklmno} = cy_i + cs_j + ca_k + olp_l + dim_m + thi_n + e_{ijklmno}$$

$$\tag{2}$$

Where;  $Y_{ijklmno}$ : least square mean of daily milk yield for calving year i, calving season j, calving age k, parity l, days in milk class m and THI class n,  $cy_i$ : effect of calving year (i=2018, 2019 and 2020),  $cs_j$ : effect of calving season (j=spring, summer, autumn and winter),  $ca_k$ : effect of calving age (k=1 for  $\leq 24$ , k=2 for 25-30, k=3 for 31-36, k=4 for 37-42, k=5 for 43-48, k=6 for 49-54, k=7 for 55-60, k=8 for 61-66, k=9 for 67-72, k=10 for 73-78 and k=11 for  $\geq 79$  month),  $olp_i$ : effect of order of lactation parity (Each animal has only one lactation record and l=1, 2, 3 and  $\geq 4$ ),  $dim_m$ : effect of days in milk class (m=1 for  $\leq 60$ , m=2 for 61-120, m=3 for 121-180 and m=4 for 180-305 days),  $thi_n$ : effect of temperature humidity index (n=48, 49, 50, 51, 52, ... and 95) and  $e_{ijklmno}$ : random residual effect.

Following this stage, the dataset used in the study was divided into two parts based on the THI=77 values (The point where milk yield starts to decline continuously), including the period before the onset of heat stress, which is referred to as the comfort period (CP), and the term covering the heat stress period (HSP). In this case, the number of daily milk yield records for CP and HSP was 123,291 and 83,712, respectively. These data sets were analyzed with the RRM (Random Regression Model) for two thermal period (CP and HSP), and the six-knot linear spline function was used in the model. Linear spline parameters are calculated between 2 knots adjacent to the milk yield record and take the value 0 among all other knots. If T is a knot vector, the parameters of the line constructed for the milk yield record at time t between knots  $T_i$  and  $T_{i+1}$  can be calculated as given in Eq. 3 and 4 (Bohmanova et al. 2008):

(3)

$$Z_i(t) = \frac{t - T_i}{T_{i+I} - T_i}$$

$$Z_{i+1}(t) = \frac{T_{i+1} - t_i}{T_{i+1} - T_i} = 1 - Z_i(t)$$
(4)

However, the parameter value for yield records at the i<sup>th</sup> knot is set to  $Z_i=1$  and  $Z_{1...i-1,i+1...q} = 0$ . For example, in an instance with six knot points, there are at least two non-zero parameter values and their sum is always equal to 1. In this study knot points were selected at the 5<sup>th</sup>, 65<sup>th</sup>, 125<sup>th</sup>, 185<sup>th</sup>, 245<sup>th</sup> and 305<sup>th</sup> days of a 305-day lactation period based on milk yields, the vector containing the function parameters that for instance include the milk yield record at t=36 calculated as follows (Bohmanova et al. 2008):

$$Z(36) = \left\{\frac{36-5}{65-5}, \frac{65-36}{65-5}, 0, 0, 0\right\} = \{0.5166, 0.4833, 0, 0, 0\}$$

As seen above, the sum of parameter values is equal to 1. In addition to this, the following RRM model (Eq. 5) is provided for estimating daily additive genetic, permanent environmental, and error variances for CP and HSP in the study:

$$Y_{tijklmnr} = cy_i + cs_j + ca_k + olp_l + dim_m + \sum_{r=l}^{6} b_r \, \emptyset_{ntr} + \sum_{r=l}^{6} a_{nr} \, \emptyset_{ntr} + \sum_{r=l}^{6} pe_{nr} \, \emptyset_{ntr} + e_{tijklmnr}$$
(5)

Where;  $Y_{tijklmnr}$ : milk yield record of cow n recorded on t within subclass calving year i, calving season j, calving age k, parity l, days in milk class m,  $b_r$ : fixed regression coefficients,  $a_{nr}$  and  $pe_{nr}$ : r<sup>th</sup> (In this study, since a six-knot linear spline function was used, six random regression coefficients for additive genetic effect and six random regression coefficients for permanent environmental effect were calculated for each animal.) random regression for animal and permanent environment effects, respectively, for animal n:  $\emptyset_{ntr}$  is the is the vector of the r<sup>th</sup> spline function for the daily record of cow n recorded on day t,  $e_{tijklmnr}$ : random residual (which was heterogeneous in this study) was calculated separately for the intervals of 5, 6-30, 31-60, 61-90, 91-120, 121-180, 181-210, 211-240, 241-270, and 271-305 days. In matrix notation, the model (Eq. 5) can be written as below (Eq.6):

$$y = Xb + Za + Wpe + e \tag{6}$$

Where; y, vector of observations (records) and b, a, pe and e: vectors of fixed, additive genetic, permanent environmental and random residual effects, respectively. X, Z and W are incidence matrices which relate records to effects.

$$\operatorname{var} \begin{vmatrix} a \\ pe \\ e \end{vmatrix} \cong \begin{bmatrix} \mathbf{G} \otimes \mathbf{A} & 0 & 0 \\ 0 & \mathbf{I} \sigma_{pe}^2 & 0 \\ 0 & 0 & \mathbf{R} \end{bmatrix}$$
(7)

The (co)variance matrices for the additive genetic and permanent environmental random regression coefficients, denoted as **G** and **P**, respectively (Eq. 7), are both  $6 \times 6$  matrices ( $\otimes$  is the Kronecker product). Additionally, **A** represents the additive genetic relationship matrix, while **I** denotes the identity matrix and  $\mathbf{R} = \mathbf{I}\sigma_e^2$ . In addition, heritability (h<sup>2</sup>) estimates were calculated based on the formula given in Eq. 8.

$$h^{2} = \frac{\sigma_{a}^{2}}{\sigma_{a}^{2} + \sigma_{pe}^{2} + \sigma_{e}^{2}}$$
(8)

Where;  $\sigma_a^2$ ,  $\sigma_{pe}^2$  and  $\sigma_e^2$  are additive genetic, permanent environmental and random residual variances respectively. The following equation (Eq.9) was used in order to obtain eigenfunctions:

$$f_i = \boldsymbol{E} \times \boldsymbol{S} \tag{9}$$

Where;  $f_i$  represents the vector containing the values of i<sup>th</sup> eigenfuctions, **E** is the matrix consisting of 6×6 eigenvector values obtained from the covariance matrix, and **S** is the 301 × 6 matrix containing the parameters of the linear spline function with six knots. In this study, to obtain least squares means using Eq. 2, SAS (2000) software was used, and for random regression analyses represented by Eq. 5 and to obtain eigenvectors of covariance matrix, WOMBAT software (Meyer 2007; Anonymous 2023b) was used.

### 3. Results and Discussion

#### 3.1. Milk yield levels for THI values

The analysis conducted using the model given in Eq. 2 in the study found that the effect of all environmental factors was

significant (P<0.01). The coefficient of determination ( $\mathbb{R}^2$ ) for the model was determined to be 0.4786, and the mean square error was 12.460.  $\mathbb{R}^2$  value, revealed that the model, which includes weather variables, explained almost half of the yield variation. Ravagnolo et al. (2000) indicated that while the moisture content in the air remained constant, the lowest humidity occurred when the temperature was at its highest. This corresponds with the findings of this study. In addition, the calculated  $\mathbb{R}^2$  value in this study is found to be higher than the values reported by Ravagnolo et al. (2000), West et al. (2003), and Freitas et al. (2006), while being very close to the values reported by Dikmen & Hansen (2009), Yazgan (2017), and Demir & Yazgan (2023).



Figure 2 - Least square means of milk yields by THI values. Dashed vertical lines show the critical THI value (72). The blue area represents the comfort period (CP), while the red area indicates the heat stress period (HSP), during which milk yield starts to decrease continuously

Figure 2 illustrates the alteration of the least square means of milk yield based on THI values. THI values ranging from 48 to 95 were calculated by taking into account the daily maximum temperature and minimum humidity values. The threshold THI value at which the milk yield started to decrease continuously was identified as 77, which was 5 units higher than the critical value (THI=72). In the previous study (Demir & Yazgan 2023), the point at which milk yield began to continuously decrease was determined to be 69, which was below the critical value (THI=72) identified in this study. The lower average milk yield of the farm where the data was obtained in this study  $(17.15\pm0.01)$  compared to the farm where the datasets used in the previous study (Demir & Yazgan 2023) were obtained (28.96±8.89) may have caused this. This difference could potentially be attributed to the greater effect on high milk-yielding cows because high milk production requires more metabolic activity and leads to an increase in body temperature (Kadzere et al. 2002; Das et al. 2016). In addition, as the THI values ranged from 48 to 77, the milk yield remained relatively stable. However, beyond this point, it began to decrease rapidly, reaching a minimum at THI=92. At THI=77, the milk yield was 18.23±0.07 kg, but when THI increased to 92, it decreased to 17.14±0.295 kg, representing a difference of 1.09 kg. According to these findings, the period from the starting THI value (48) in Figure 2 to the vertical line (77) and the blue area shown can be considered as the comfort period (CP), while the period after the vertical line and shown in red represents the heat stress period (HSP). Additionally, as can be observed from Figure 2, minor fluctuations in milk yield are observed during the CP. The fluctuations observed during the CP period may be attributed to the utilization of fans, shading, and sprinkler equipment, which may reduce the heat stress at higher temperatures. This can cause the THI to exhibit not only a linear but also a zigzag pattern, as shown in Figure 2.

#### 3.2. Variance components and heritability values for CP and HSP

The daily estimated additive genetic, permanent environmental, random residual error and phenotypic variances for CP and HSP are shown in Figure 3. According to this, all variance components except for random residual variance exhibited a fluctuating trend throughout lactation for both CP and HSP. The highest error variance values for both CP and HSP were determined to be  $1.5284 \text{ kg}^2$  and  $1.5228 \text{ kg}^2$ , respectively, between the  $30^{\text{th}}$  and  $60^{\text{th}}$  days. Afterwards, from day 121 until the end of lactation, the random error variance values for both CP and HSP tended to be close to each other and remained constant, and did not exceed the value of  $0.35 \text{ kg}^2$  for either. When examining the daily changes of the additive genetic variance during lactation for both thermal periods (CP and HSP), intervals were observed where one was higher or lower than the other (Figure 3). Additive genetic variance values for HSP reached their highest value on the  $66^{\text{st}}$  day of lactation ( $8.57 \text{ kg}^2$ ). In contrast, the highest estimated additive genetic variance value for CP was reached on the  $125^{\text{st}}$  day of lactation and was only predicted as  $3.06 \text{ kg}^2$ . In other words, additive genetic variance increased during the heat stress period, which is consistent with the findings of Armstrong (1994), Ravagnolo & Misztal (2000), Aguilar et al. (2009) and Bernabucci et al. (2014).



Figure 3 - The daily estimated additive genetic, permanent environmental, random residual error, and phenotypic variances for CP ( — ) and HSP ( — )



Figure 4- Lactation curve plotted from 207 003 daily milk yield records used in the study

As shown in Figure 3, the highest additive genetic variance value  $(8.57 \text{ kg}^2)$  for HSP was obtained on the 66<sup>th</sup> day of lactation under heat stress conditions, whereas the value for CP was only 1.56 kg<sup>2</sup> on the same day. As can be observed from the lactation curve given in Figure 4, the highest additive genetic variance was obtained during the peak milk yield period. The difference in milk yield between genetically tolerant and sensitive cows to heat stress may have become more pronounced during this period, and this could have led to an increase in the additive genetic variance. The fact that the difference in additive genetic variance between CP and HSP during the lactation period is not as high as around the 66<sup>th</sup> day at any other period further confirms this result.

As can be observed from Figure 3, the highest permanent environment variance value (18.39 kg<sup>2</sup>) for HSP was obtained on the 121<sup>th</sup> day of lactation under heat stress conditions, whereas the value for CP was only 9.75 kg<sup>2</sup> on the same day. While permanent environment variance values for HSP reached their highest value on the 121<sup>st</sup> day of lactation, the highest estimated permanent environment variance value for CP was reached on the 61<sup>st</sup> day of lactation and was only predicted as 10.86 kg<sup>2</sup>. The estimated permanent environmental variance for HSP was found to be consistently higher than the permanent environmental

variance estimated for CP throughout much of the lactation period. This suggests that, similar to additive genetic variance, permanent environmental variance also increases under heat stress conditions. These results are consistent with the findings of Ravagnolo & Misztal (2000) and Aguilar et al. (2009).

As previously stated, the permanent environmental variance for HSP reached its highest value on day  $121^{\text{th}}$ , which coincided with the end of the calving-to-conception interval (days open) at the farm where the study was conducted. Towards the end of this period, estrus continues in cows, and sudden drops in milk yield are observed (Rearte et al. 2018). In this case, the negative effect of heat stress may have also contributed to the increase in permanent environmental variance, in addition to the decrease in milk yield caused by estrus. This is because, as can be observed from Figure 3, on the days when the permanent environmental variance for HSP reached its highest value, the estimated permanent environmental variance for CP was only 9.75 kg<sup>2</sup> as previously mentioned. Abeni et al. (2007) demonstrated that the alterations in blood parameters associated with energy balance and enzyme activity during heat stress were most pronounced in cows in midlactation. Additionally, Perera et al. (1986) reported that the negative impact of summer heat stress on milk yield in cows was most significant during midlactation. These factors could provide another reason for why the permanent environmental variance increases towards the midlactation period during the heat stress period. Also, shown in Figure 3, on the days when permanent environment variance was highest, phenotypic variance also reached its highest value (19.88 kg<sup>2</sup>). Additionally, phenotypic variance is higher for HSP compared to CP, except for short periods that cover the beginning and end of lactation for HSP.



Figure 5 - The daily estimated heritability values for CP and HSP

Daily changes in the heritability estimates are presented in Figure 5. When considering changes in heritability in thermal zones, both CP and HSP exhibit fluctuating values. The daily course of heritability values for both HP and CP showed a significant similarity with the course of additive genetic variance values as expected (Figure 3 and 5). The heritability of HSP was found to increase above 50% on the  $66^{th}$  day in accordance with the trend of the additive genetic variance, with an average of  $0.18\pm0.010$  throughout lactation. On the other hand, the average heritability estimate for CP was calculated as  $0.13\pm0.007$ . However, the highest heritability for CP was reached around the  $120^{th}$  day (0.23). In other words, the highest heritability for CP was reached around the  $120^{th}$  day (0.23). In other words, the highest heritability for CP was reached around the  $120^{th}$  day (0.23). In other words, the highest heritability for CP was reached around the  $120^{th}$  day (0.23). In other words, the highest heritability for CP was reached around the  $120^{th}$  day (0.23). In other words, the highest form 0.10 to 0.24, which is slightly broader than the range observed in this study. Similarly, Ravagnolo & Misztal (2000) estimated heritability values between 0.16 and 0.21 when THI values ranged from 72 to 85. In our research, since multiple lactation records were not available for each animal, it was not possible to estimate how much the variance components changed by lactation order. This difference may explain the variation between the studies. Other factors that could contribute to the inconsistency include the use of daily milk yield records in this study versus monthly test-day milk yield records in the studies, as well as potential variations in the number of knots used in the linear spline models, differences in cooling applications such as fans and water spraying and other metrological factors.

# 3.3. Eigenfunctions for CP and HSP

Eigenfunctions, derived from the eigenvectors of the genetic (co)variance matrix (Kirkpatrick et al. 1990), can offer a perspective on the impacts of selection throughout the lactation period. Eigenvalues with their relative proportions (%) of coefficient matrix of the additive genetic covariance functions for CP and HSP are presented in Table 3. For CP and HSP, the first eigenvalues of the coefficient matrices of the additive genetic covariance functions accounted for approximately 63.76% and 69.93% of the total eigenvalues, respectively, in the six-knot linear spline model. The fourth, fifth, and sixth values can be neglected in contrast to the first three, which have negligible proportions of the total eigenvalues related to the variation in additive genetic variance. The eigenfunctions of the additive genetic coefficient matrix of the covariance functions for both CP and HSP in the six-knot linear spline model were plotted in Figures 6 and 7. Considering CP, the first eigenvalue of the coefficient matrix of the additive

genetic covariance function accounted for approximately 63.76% of the total eigenvalues. The large first eigenvalue implies that selecting based on the first eigenfunctions will lead to rapid changes in the lactation curve. This suggests that genetic selection for the 121<sup>st</sup> day would be successful. As previously mentioned, for CP, this period corresponds to the end of the estrous cycle, and it is the point where the eigenfunctions reaches its highest value, similar to the case of permanent environmental variance (Figure 3 and 6). However, when considering HSP, the first eigenvalue of the coefficient matrix of the additive genetic covariance function accounted for approximately 63.76% of the total eigenvalues. Similarly, the large first eigenvalue suggests that selection based on the first eigenfunctions will result in rapid changes in the lactation curve. Additionally, for HSP, the eigenfunctions value reaches its highest point on the 60<sup>th</sup> day. This implies that genetic selection around the 60<sup>th</sup> day would be successful. As discussed previously, it was the period around the 60<sup>th</sup> day when the peak milk yield is achieved, and for HSP, it coincides with the time when both the genetic variance and heritability were at their highest.

# Table 3- Eigenvalues and their relative proportions (%) in the coefficient matrix of additive genetic covariances for CP and HSP.

		Order of eigenvalues					
Thermal period	-	1.	2.	3.	4.	5.	6.
СР	Eigenvalue	5.61	2.02	1.14	0.02	0.00	0.00
	(%)	63.76	23.03	12.93	0.27	0.02	0.01
HSP	Eigenvalue	13.08	4.66	0.77	0.20	0.00	0.00
	(%)	69.93	24.89	4.10	1.06	0.01	0.00



Figure 6- First three eigenfunctions (EF) of the random regression genetic covariance matrix of lactation for CP



#### Figure 7- First three eigenfunctions (EF) of the random regression genetic covariance matrix of lactation for HSP.

In this study, due to the unavailability of multiple lactation records for each animal, the relationship between the lactation order and heat stress could not be examined. Similarly, the relatively low number of dairy cows with multiple offspring prevented the inclusion of maternal effects in the model. In the future, as global warming's negative effects on dairy animals' yields are expected to increase, the selection of genetically heat-tolerant dairy cattle may require the use of more complex models. It can be said that similar studies should continue using more complex models that include multiple genetic effects (such as direct and maternal genetic effects, genetic groups, etc.) with larger datasets containing more lactation records.

# 4. Conclusions

This study demonstrated the impact of heat stress specifically on additive genetic variance and permanent environmental variance as it differs from other studies by using daily milk yield records for estimating variance components. Heat stress resulted in an increase in additive genetic, permanent environmental, and consequently, phenotypic variance in this research. Although variance ratios may vary depending on the herd and region, it does not change the fact that heat stress will affect genetic parameters. According to the findings obtained from the research, it has been concluded that the time periods for selection should coincide with the period when peak milk yield is achieved under heat stress conditions, whereas for the period without heat stress, it should be around the 120<sup>th</sup> day of lactation. These results indicate that climatic factors such as temperature and humidity should be included in the models used for genetic parameter and breeding value estimation. Thus, it may be possible to identify dairy cattle that are genetically more tolerant to hot conditions. In this way, more successful outcomes can be achieved in selection studies. Additionally, similar studies should be replicated in hot-dry regions with different dairy cattle breeds and larger herds to obtain data that can further validate the findings of this study.

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