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Reaction norm models to quantify the genetic basis of heat stress tolerance in Spanish Holstein.

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Summary

In the present thesis the effect of heat stress on milk, fat and protein yields and somatic cell score (SCS) has been studied in the Spanish Holstein breed of dairy cattle. In this work there are two levels of analysis; the first one is a phenotypic analysis carried out to determine the climatic variables most correlated to the production traits and to estimate the tolerance thresholds and slopes of the responses of the dairy traits and SCS studied to heat stress and to determine the reaction norm model to be used in analyses of tolerance to heat stress in Spanish Holstein. The second one is a genetic analysis of formerly cited responses, undertaken with the objective to estimate the environmental and genetic (co)variance components of heat stress tolerance. A total of 2,514,762 test-day records belonging to 128,112 lactating goats distributed in 468 herds. Whereas that for genetic analysis used a random sample of 259,667 test-day records belonging to 27,377 cows distributed in 123 herds collected between 2002 and 2012, combined with maximum and average temperatures and the values of an index of temperature and relative humidity (THI), registered the day of milk recording in meteorological stations located less than 30Km from the farms. For the first study, a Ridge regression analysis and a BLUP method were carried out in order to select the climatic variables and dates that were recorded, having the highest correlations with the dairy traits and SCS under study. Then, tolerance thresholds and slopes of the regressions of these traits with the selected climatic variables were estimated with spline and polynomial models by means of Bayesian methods. Results shows that increases in temperature have been associated to a decrease in production traits and Heat tolerance thresholds have been found to be higher for milk (around 29.2ºC) and SCS than for fat and protein yields (around 18.1ºC). Polynomial functions showed better convergence behaviour and provided better goodness of fit than the classical splines model used to model heat stress. Genetic variability for response to increases in heat load has been observed. However, this variability may be mainly associated to level of production, so that animals with higher production levels seem to be more prone to suffer heat stress. Finaly, A cubic polynomial seems to provide better quality of adjustment than quadratic polynomial to determine both the population and individual deviations associated to increasing heat loads

Resumen

La presente tesis trata del efecto del estrés térmico sobre las producciones de leche, grasa y proteína y sobre la puntuación de células somáticas (SCS) de la raza frisona española. En este trabajo hay dos niveles de análisis, a nivel poblacional objetivo fue determinar las variables climáticas más correlacionadas con los caracteres productivos y SCS y estimar el umbral y la pendiente de la tolerancia de la respuesta al estrés térmico de dichos caracteres y determinar el modelo de la norma de reacción para usarlo posteriormente en los análisis de tolerancia al estrés térmico. A nivel individual se hecho un análisis genético realizado con el objetivo de estimar los componentes de (co)varianza genéticos y ambientales de la tolerancia al estrés térmico. Para llevar a cabo los análisis fenotípico 2,514,762 registros del día de control de la producción y composición de la leche tomados de 128,112 vacas distribuidas en 468 rebaños. Mientras que para los análisis genéticos una muestra aleatoria ha cogido de 259,667 registros del día de control de la producción y composición de la leche tomados de 27,377 vacas distribuidas en 123 rebaños. Se combinaron con los datos de temperatura máxima y media y un índice de temperatura y humedad relativa (THI), registrados el mismo día del control lechero, en estaciones meteorológicas ubicadas a menos de 30 Km de cada explotación. En este primer estudio, se utilizaron los métodos de regresión "Ridge" and BLUP para seleccionar las variables climáticas, y las fechas de registro de las mismas, más correlacionadas con los registros de los caracteres lecheros en estudio. Después, se estimaron el umbral y la pendiente de la respuesta de los caracteres lecheros a cada una de las variables climáticas seleccionadas anteriormente, mediante modelos mediante modelos lineales y Polinómicos utilizando para ello métodos Bayesianos. Los resultados muestran que los aumentos de temperatura se han asociado a una disminución en los caracteres de producción and los umbrales de tolerancia de calor se han encontrado más alto para la leche (alrededor de 29,2 ° C) y SCS que para grasa y proteína (alrededor de 18,1 ° C). Funciones polinómicas mostraron un mejor convergencia y previsto una mejor bondad de ajuste que el modelo splines clásico. La variabilidad genética de la respuesta al aumento de la carga de calor ha observado. Sin embargo, esta variabilidad puede ser en gran asociado con el nivel de producción de

manera que los animales con niveles de producción más altos parecen ser más propensos a sufrir el estrés por calor.

Al final, Un polinomio cúbico parece proporcionar una mejor calidad de ajuste del polinomio cuadrático determinado que tanto la población como las desviaciones individuales asociados al aumento de las cargas de calor.

1.Introduction

1.1. Climate change and dairy cattle in Spain

Increases in global average air and ocean temperatures, widespread melting of snow and ice as well as rising global average sea level indicate that global warming is unequivocally inevitable (Intergovernmental Panel on Climate change (IPCC, 2007). This Climate change is expected to have an impact on animal production throughout the world (IPCC, 2007). IPCC (2007) reported that increased droughts and heat waves, especially during summer, are likely to dominate climate change impacts in Southern and Eastern Europe, while increased flooding and water logging in winter may dominate climate change impacts in the Northern part of the Mediterranean basin.

In Spain, temperatures, especially minimum temperature, have increased over the last century by around 1.5ºC the annual average (Fernández-González *et al*., 2005). Climate change has had a particularly marked effect on southern Spain, increasing temperatures and reducing rainfall. In future perspectives, the analysis indicated that Spain, southern France and Italy should be expected to undergo the highest THI increase, which in 2041 - 2050 will range between 3 and 4 units. The area presents characteristics indicating risk of thermal stress for farm animals during summer months. The authors claim that at the end of the 2050, only northern Spain, France and Alpine regions are expected to have mean values of summer THI below the upper critical value of 68 (Segnalini *et al.*, 2013). Meanwhile, IPCC (2007) predicted an average rise in air temperature ranging from +2 °C to +6.5 °C by the end of the century, which is slightly higher than the world average increment estimated to range from +1.1 °C to +6.4 °C.

Holstein is a dominant dairy breed in the world because of its high milk production. Although most of Holsteins are bred in temperate or cold parts of the world (Europe, Canada and USA), they are also raised in hot and tropical environments, where they are challenged by many factors, including heat stress, which reduces production and fertility and can even cause death. It is assumed that also in regions traditionally characterized by less extreme climate conditions, cows will be faced with temperatures beyond their »comfort zone« (IPCC 2007).

In dairy cattle, especially in Spanish Holstein is raised in all the country as showed Table 1.1. Andalucía and Castilla la Mancha have a significant population Compared to the rest country and detected many cows in these community. For

example, in 2012 detected 36,531 and 17,357 cows in Andalucía and Castilla la Mancha, respectively (Table 1).

Table1.1. Distribution of Spanish Holstein in Spain by community (Ministerio de Agricultura, Alimentación y Medio Ambiente, 2012)

	Total Reproductive		Total animals		Total	Nº of farms
	dam	sire	dam	sire		
Andalucía	**23,759**	**172**	**36,531**	**236**	**36,767**	**162**
Aragón	7,156	5	10,990	6	10,996	20
Cantabria	48,690	268	71,758	384	72,142	895
Castilla La Mancha	**11,193**	**38**	**17,357**	**51**	**17,408**	**89**
Castilla León	45,694	101	70,409	132	70,541	433
Cataluña	49,899	488	75,886	552	76,438	328
Comunitat Valenciana	3,582	1	5,495	1	5,496	7
Extremadura	226	0	331	0	331	3
Galicia	193,884	593	298,716	759	299,475	3,591
Illes Balears	12,546	213	16,379	223	16,602	188
Madrid	4,663	12	6,946	14	6,960	29
Navarra	19,657	66	29,205	76	29,281	149
País Vasco	21,612	72	32,181	100	32,281	302
Principado De Asturias	54,095	382	77,942	489	78,431	1,098
Total	496,656	2,411	750,126	3,023	753,149	7,294

1.2. Heat stress in Dairy cattle

Stress produces with certain magnitude of external forces that are able to displace the bodily system from its resting or ground state (Yousef, 1985). Stress produces with certain magnitude of external forces that are able to displace the bodily system from its resting or ground state (Yousef, 1985). Many sources of stress, such climatic, as extensive cold and heat, nutritional, due to feed or water deprivation; social,

resulting from a low rank in the pecking order or internal, due to some physiological disorder, pathogens or toxins (Stott, 1981). Strain is the displacement from the resting or ground state by internal forces (Yousef, 1985). Once, any combination of environmental factors cause the effective temperature of the environment to be higher than the animal's thermoneutral zone, the heat stress is achieved (Armstrong, 1994).

For animal, reactivity with best performance and minimum of metabolic rate coincide with a range of environmental temperature that is named Thermoneutral zone.

Temperatures above the thermoneutral zone trigger a chain of physiological, anatomical and behavioral changes in the animal's body, such as reduction of feed intake, decline of performance (milk production, growth, and reproduction), decrease of activity, increase of respiratory rate and body temperature, increase of peripheral blood flow and sweating and change in endocrine function. Heat stress affects animal performance and productivity at all stages of the life cycle. Animals do not maintain strict homeothermy when there are heat stress (Silanikove, 2000).

1.3. Heat stress impact on milk production

Several studies reported that heat stress in dairy cattle affects production (Maust *et al*., 1972, Fuquay, 1981, Bryant *et al*., 2007b) and reproduction (Ravagnolo and Misztal, 2002, Garcia-Ispierto *et al*., 2007). Economic losses due to heat stress for the U.S. dairy industry are estimated to be between \$897 and \$1500 million dollars per year (St-Pierre *et al*., 2003).

For lactating dairy cows, the ambient temperatures above 25 °C are associated with lower feed intake, drops in milk production and reduced metabolic rate, as reported by Berman (1968). Critical maximum temperature for cows is assumed to be at the level of 25-26°C (West, 2003) or 24-27°C (Brouček *et al.,* 2009). Milk yield decreases of 10 to 40% from winter to summer have been reported for Holstein cows (Du Preez *et al.*, 1990b). At 29 °C and 40 % relative humidity, Bianca (1965) determined decreases of daily milk yield of 3, 7 and 2 % in Holstein, Jersey and Brown Swiss cows, respectively. Additional decreases of milk yield of 31, 25, and 17 % were observed in former breeds when relative humidity increased up to 90 %.

Ravagnolo *et al.* (2000) stated that milk yield appears relatively constant until about 24°C and then declines at a pace of 0.2 kg per unit increased in THI when THI exceeded 72. Under Mediterranean climatic conditions, Bouraoui *et al.* (2002) reported drops in milk yield of 0.41 kg per cow and day for each point of increase in the value of THI above 69. Ravagnolo *et al.* (2000) and Bouraoui *et al.* (2002) estimated a negative correlation (r = -0.76) between milk yield and THI. Besides, they referred that as the THI values increased from 68 to 78, milk production decreased 4 kg. Gantner *et al.* (2006) indicated that milk production decreased as THI increase and the most intensive decrease took place between 60 and 120 days of lactation. A highly significant decrease of daily milk yield due to high THI values was also observed in heifers and cows by Gantner *et al.* (2011).

West *et al.* (2003) mentioned that during hot weather, the mean THI registered two days earlier than test day had the greatest effect on milk yield. Milk yield of Holsteins declined 0.88 kg per each unit of the THI registered two days before the day of milk recording. Herbut and Angrecka (2012) recorded a decrease in milk production 4 days after starting to register high temperatures. Production decrease varied from 0.18 to 0.36 kg per THI unit depending on the level of milk production of the cow. Coppock *et al.* (1982) concluded that high-producing cows are more affected by heat stress than low-producing cows. An increase of milk yield increases the sensitivity of cattle to thermal stress and reduces the "threshold temperature" at which milk losses occur (Berman, 2005). Johnson *et al.* (1988) found a higher average decline of persistency in cows yielding more than 30 kg/day (-0.059 % per day) compared with cows yielding less than 25 kg/day (-0.019 % per day). Similarly, Berman (2005) reported a drop of 5°C in the threshold temperature for heat stress when milk production increased from 35 to 45 kg/day.

In sheep and goat, Menéndez-Buxadera *et al.* (2012b) reported a decline in sheep milk production at THI=45 and that decline reached 98 g/day every increase of 5 degrees in THI. However, Sevi *et al.* (2001) reported a reduction of milk yield after ewe exposure to temperatures over 35 ºC, even for short periods of time. Menéndez-Buxadera *et al.* (2012a) observed genetic variation for heat stress tolerance in Murciano-Granadina and Payoya goats which, according to these authors, could be used for selection purposes. Finocchiaro *et al.* (2005) reported that the greatest decrease in

daily milk yield 62.2 g (-3.9%) per unit of THI above a threshold value of THI=23 was observed for temperature and humidity records taken the day before milk recording in sheep

1.4. Ways of measuring heat stress

The main factors which are responsible for energy flow to the animal are: effective air temperature, solar radiation, relative humidity, wind speed and structural properties of animal's coat (Yousef, 1985). Thermal environment can be represented by a single or a combination of the bioclimatic factors. Extensive efforts have been undertaken to develop an index to take into account all environmental factors (ambient temperature, relative humidity, solar radiation and wind speed) causing measurable physiological responses.

Many indices combining different environmental factors to measure the level of heat stress have been proposed. However, the lack of data, publicly available, on the amount of thermal radiation received by the animal, the wind speed, precipitation and rainfall limited their use. Therefore, the majority of studies on heat stress in livestock have focused mainly on temperature and relative humidity (Igono *et al*., 1985; Igono and Johnson, 1990; Ravagnolo and Misztal, 2000; Bouraoui *et al*., 2002; St-Pierre *et al*., 2003; West, 2003; Correa-Calderon *et al*., 2004) because data on the amount of thermal radiation received by the animal, wind speed, and rainfall are not publicly available. On the other hand, temperature and humidity records can be usually obtained from a meteorological station located nearby. To indicate the degree of stress and to determine the influence on dairy animal a temperature-humidity index (THI) was developed that combines the effects of air temperature and humidity is the most common indicator for heat stress (Bianca, 1962; NRC, 1971). Marai *et al.* (2007) showed that the severity of heat stress is correlated to both ambient temperature and humidity level and the effect of heat stress is aggravated when high temperature is accompanied with high ambient humidity.

THI was originally developed by Thom (1958) as an index to measure the levels of discomfort of humans during summer months. Afterwards, its use was extended to bovines by Johnson *et al.* (1961). This index has been developed as a weather safety index to monitor and reduce heat stress related losses.

The Table 1.2 showed the different calculation methods of THI developed over the years, Depending on the author, formulas are based on different weightings of dry bulb temperature (Tdb) and air moisture. THI integrate air moisture in the index by means of the relative humidity (RH), which provides information about water saturation of the air at a given temperature (Kelly and Bond, 1971; NOAA, 1976; LPHSI, 1990; Finocchiaro *et al.*, 2005; Mader *et al.,* 2006). Others use wet bulb temperature (Twb), which represents the equilibrium temperature of a thermometer covered with a cloth that has been wetted with pure water (Thom, 1959; Bianca, 1962; NRC, 1971) or dew point temperature (Tdp), the temperature to which the air must be cooled for saturation to occur; that is, the temperature at which RH is 100% (NRC, 1971; Yousef, 1985).

Table 1.2. Formulas to calculate Temperature Humidity Index (THI).

Formula	Reference
THI1= [0.4 × (Tdb °C + Twb°C)] × 1.8 + 32 + 15	Thom (1959)
THI2= (0.35 × Tdb °C + 0.65 × T wb°C) × 1.8 + 32	Bianca (1962)
THI3= (0.15 × Tdb °C + 0.85 × Twb°C) × 1.8 + 32	Bianca (1962)
THI4= Tdb °C+(0.36*Tdp°C)+41.2	Yousef (1985); Bosen (1959) and Kibler (1964)
THI5= (0.55*Tdb °C +0.2*Tdp°C)*1.8+32+17.5	NRC (1971)
THI6= (Tdb °C + Twb°C) × 0.72 + 40.6	NRC (1971)
THI7= Tdb °F-[(0.55-0.55*RH)*(Tdb °F-58)]	Kelly and Bond (1971); NOAA (1976) and LPHSI (1990)
THI8= Tdb °C -[0.55*(1-RH)]*(Tdb °C − 14.4)]	Finocchiaro *et al.* (2005)
THI9= (0.8*Tdb°C)+((RH)*(Tdb °C-14.4))+46.4	Mader *et al.* (2006)
THI10= Tdb °C –[(0.31 − 0.31 RH)(Tdb °C − 14.4)]	Marai *et al.* (2007)

T_{db}: dry bulb temperature; T_{wb}: wet bulb temperature; T_{dp}: dew point temperature; RH: relative humidity.

1.4. Diminishing the impact of heat stress

Many strategies developed for reduction of effects of heat stress on animal agriculture, such as cooling systems and barn construction and facilities, changes in feed and its management (Armstrong, 1994; West, 1999; Berman, 2008; Stowell et al., 2009).

Same with the environmental management, la production still decreases during the summer months with some facets of production, especially reproduction; also, the strategies can be successful in the short-time and during a chronic stress, the depressed effect of thermal stress cannot be totally eliminated, beside, taking account to knowledge of associations between economics, climate and genetic in terms of production or fertility, in addition, current selection acts against heat tolerance, so, the breeding strategies for heat tolerance in dairy cattle may be adequate in the long-term and economically.

Objective

Determine the importance of heat stress on production traits and SCS and the genetic components of these traits.

2. Materials and Methods

2.1. Production and meteorological databases

Initial data bases

Data were obtained from two sources of information. The first one was the Confederation of Associations of Spanish Friesian (CONAFE) that provided records of phenotypic observation of production traits and the pedigree file. The second one was the meteorological state agency (AEMET) that records meteorological data.

The raw database of Spanish Friesian test-day records for milk, fat and protein yield and somatic cell count (SCC) from up to 17 parities provided by CONAFE comprised 7,347,494 test-day records of 209,844 of Spanish Friesian cows collected from 1996 through 2012 in 568 herds in Castilla la Mancha (CLM) and Andalucía (AND), with an average of 10 test-day records per lactation and 2.50 lactations per cow. Concerning the weather stations, hourly (if available) or daily temperature and relative humidity data from 718 stations in both regions (CLM and AND) were provided by AEMET. Maximum (TMAX) and average (TAVE) daily temperatures together with two indices, that combine daily average (THIAVE) and maximum temperature (THIMAX) with relative humidity, respectively. THI indices were calculated according to the following formula (NRC, 1971)

$$THI = (1.8*T+32)-(0.55-(0.55*HR/100))*(1.8*T-26),$$

where, T is temperature in Celsius degrees and RH is relative humidity, expressed as a percentage. Table 2.1 shows the number of meteorological stations by region and the type of collected information.

Table 2.1. Number of stations collecting hourly or only average temperature and humidity by region.

	Andalucía	Castilla la Mancha
Daily average temperature only	572	146
Hourly temperature	134	55
Daily/hourly humidity	154	51

Thereafter, herds were matched with the closest weather station, based on minimum distances from latitude and longitude information using a suit of R programs. Herds farther than 30 km from their closest weather station were discarded.

Production data edits

Upon receipt of the raw data, a series of analyses to remove anomalous data were performed. Firstly, data with parity number exceeding five were removed because parities over this number are rare in this population. Records with missing data of milk and missing test-day date were removed. Records obtained before 2002, were also removed. Data above the 99th percentile and below the 1st percentile of the distribution of each character and days in milk (DIM) per control and parity number (shown in Tables 2.2 and 2.3) were discarded. Moreover, 2308 and 4810 test day records obtained at extreme temperatures TAVE and TMAX, respectively, were ignored to avoid problems in the subsequent adjustment of the production vs. temperature curves. Finally, following CONAFE's edits for genetic evaluations, all records from parities outside the age intervals shown in Table 2.4 were deleted

Table 2.2. Thresholds by number of control to consider valid test-day records for production traits for all lactations.

Number of control	Milk (kg)	Fat (%)	Protein (%)	Days in milk
1	8.6-55.5	1.60-6.47	2.46-4.49	4-63
2	12.0-59.0	1.54-5.60	2.39-3.83	33-95
3	13.0-57.5	1.54-5.50	2.45-3.86	63-126
4	12.3-55.0	1.56-5.49	2.52-3.94	93-157
5	12.0-52.5	1.58-5.51	2.59 4.00	123-189
6	11.0-50.0	1.60-5.55	2.64-4.07	154-220
7	10.0-48.0	1.67-5.60	2.68-4.15	184-251
8	8.0-45.6	1.73-5.69	2.73-4.27	214-281
9	8.0-44.0	1.81-5.75	2.77-4.37	245-312
10	7.0-42.0	1.87-5.81	2.80-4.49	275-343
11	6.5-41.0	1.92-5.84	2.83-4.55	305-375
12	6.0-40.0	1.96-5.90	2.85-4.63	335-418
13	6.0-39.7	1.99-5.92	2.86-4.68	366-451
14	6.0-39.0	2.03-5.93	2.88-4.72	396-484

Table 2.3. Limits for valid somatic cell counts (SCC) per parity.

	Parity						
	1	2	3	4	5	6	7
SCC	4-3406	7-4846	9-5362	6-5800	1-5655	5-5753	1-6517

Table 2.4. Age intervals required for valid parity records.

Parity number	Age at calving (months)
1	18-40
2	28-59
3	38-77
4	48-94
5	58-110

The file obtained after these edits contained 2,514,762 test-day records until the fifth lactation, between 4 and 484 DIM for 128,112 cows from 468 herds and years 2002-2012. The distribution of records, cows and average number of controls per lactation can be seen in Table 2.5.

Table 2.5. Spanish Holstein cows and records number and mean of control number per lactation number.

Lactation number	Cows number	Test-day records	Mean of control number
1	99,593	932,506	9.3
2	78,422	700,244	8.9
3	52,635	452908	8.6
4	32,514	276,968	8.5
5	18,133	152,136	8.3

Because of the extremely skewed distribution of SCC, somatic cell score (SCS) was used in the subsequent analyses. SCS was calculated according to the formula (Ali and Shook, 1980):

$$SCS = \log_2 (SCC/100) + 3,$$

Date of weather recording

After editing the data, a multiple regression analysis of each character on daily temperatures of milk recording and the previous 15 days was carried out to determine the day that showed the highest regression coefficient. Data were pre-adjusted to correct noise factors that could distort the posterior estimate of the relationship between temperature and milk production. For the pre-correction, solutions obtained by a BLUP method under the following model were used:

$$y_{ijkl} = HY_i + LADIM_j + a_k + e_{ijkl,} \qquad [1]$$

where,

y_{ijkl} = observations on production traits and SCC

HY_i = herd-year of calving fixed effect (3,712 levels)

$LADIM_j$ = lactation-age at calving-days in milk fixed effect (533 levels)

a_k = animal random effect, with a_k i.i.d. $N(0, \sigma_a^2)$ (128,112 levels)

e_{ijkl} = residual effect, with e_{ijkl} i.i.d. $N(0, \sigma_e^2)$

In this step we used the programs BLUPf90 (Misztal, 1999). Please, notice that no pedigree information was used in the pre-correction (the relationship matrix of random animal effect was not considered in the analysis of pre-correction using mixed model).

After adjusting the mentioned factors, a multiple regression analysis was performed using a ridge regression procedure to avoid the problems of colinearity between regressor variables (temperatures on consecutive days) using the library MASS of R (R3.2 version 2.15.2).

2.1.Reaction norm of production to temperature increase. Population level.

The aim of this part was to determine the threshold and slope of the response of production traits to temperature increases. The edited data base described in the previous section was used in this study.

Statistical models

Two types of models were used, a splines model (SP) and Legendre polynomial models (LP). In SP models, two splines are fitted. The first corresponds to the comfort region, where production or somatic cell score (SCS) remain unaffected by temperature, and the second describes the stress region, where production and SCS follow a linear decay or increase with temperature. The point where the two splines meet is the thermotolerance threshold. For LP, quadratic and cubic functions were fitted to define the pattern of response to increasing temperatures. The general model used to determine the effect of temperature on productive traits and SCS at the population level had the following general form:

$$y_{ijkl\_T} = HY_i + LADIM_j + f(T) + a_k + e_{ijk\_Tl,}$$

where, y_{ijkl_T}, is the observation for a given trait at a certain temperature, T, HY_i, $LADIM_j$;, and a_k are the same as in model [1] used in the date of weather recording section; $f(T)$ is a function of temperature (T= TAVE or TMAX), that differs between SP and LP models.

For SP models

$$f(T) = \begin{cases} 0 & \text{if } T \leq T_o \\ b(T - T_o), & \text{other} \end{cases},$$

where, b is the slope of response to temperature increases and T_o is the thermotolerance threshold. The aim of the SP models was the estimation of T_o, b.

For LP models,

$$f(T) = \sum_{i=0}^{q} b_i Z_i(T),$$

where, b_i are the regression coefficients, $Z_i(x(T))$ are the covariates of the Legendre polynomials of order q (in our case, q=3), evaluated at the corresponding standardized values of the T variables in the interval [-1, 1], x(T).,

x= -1

where, tmin was the minimum temperature or THI and range was the range temperature or THI.

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In the PL models, the maxima and derivatives (slopes) at subsequent temperatures or THI of the LP functions were obtained as proxies for the threshold and slope in the SP models

The inferential method implemented for estimating parameters for SP and LP models was a Bayesian Markov-chain Monte Carlo procedure, specifically a Gibbs sampler algorithm. For the SP models, a Metropolis Hasting sampling within the Gibbs sampler was used to obtain values for the threshold To, which did not have a known conditional distribution. A software program written in Fortran90 language provided by J.P. Sánchez (personal communication) was used in the estimation of the unknowns in the SP models. For the LP models, the Gibbsf90 package (Misztal, 1999) was used. A single long chain of 3,000 samples was generated for both models. The first 1,000 samples were discarded as a burn-in, and the remaining samples were used to compute posterior means of model's parameters. Convergence of Gibbs chains was monitored by visual inspection of plots of samples and the Geweke criterion. Post-Gibbs analysis was performed using boa package of R (R3.2 version 2.15.2). For each parameter, the mean and the high posterior density intervals (HPD95%) were calculated.

Overall, six models, one SP and two LP models (quadratic and cubic) using either TAVE or TMAX, were fitted for each trait. Models were compared according to their goodness of fit, measured through the Deviance Information Criterion (DIC, Spiegelhalter et al., 2002).

2.2. Reaction norm of production to temperature increase. Individual genetic and environmental components

Material:

In order to obtain a more tractable data from a computation point of view, a random sample of 25% of the herds were used in this part. From all records in the sampled herds, first lactation records were then extracted to avoid the complexity of models including test-day records from different lactations. The data set used in this part of the study contained 259,667 test-day records including production traits and somatic cell score (SCS) for 27,377 Spanish Holstein cows in 123 herds. Table 10 shows a summary of the basic statistics of the sample and total data sets.

Table 2.6. Averages and first and 99th percentile for the sampled and total (in parenthesis) data sets.

	Average	1-99 Percentiles
Milk yield (kg/d)	28.20 (28.27)	6.00-58.40 (6.00-59.00)
Fat (%)	3.53 (3.57)	1.54-6.46 (1.54-6.46)
Protein (%)	3.28 (3.27)	2.39-4.71 (2.39-4.72)
Fat yield (kg/d)	0.97 (0.99)	0.97-2.70 (0.13-2.85)
Protein yield (kg/d)	0.91 (0.91)	0.18-2.01 (0.17-2.16)
SCC (thousands/ml)	170 (169)	1-4037 (1-4037)

As shown in Table 2.6, sampled and total data showed similar averages and range for all traits.

A Pedigree file was constructed using genealogical information provided by CONAFE . Ancestors of the animals with data were traced back for three generations (until the great grandparents). The final genealogical file contained 54,173 animals.

Statistical models:

A test day type of model that included a random regression on DIM and a random reaction norm to temperature were fitted following Brügemann *et al*. (2011) and Hammami *et al*. (2013). Only LP models were considered in this part because of the poor convergence behavior observed for the SP models in the previous part.

The general model equation was:

$$y_{ijkl\_DT} = HYS_i + ADIM_j + \sum_{r=0}^{3} b_r Z_r(T) + \sum_{r=0}^{3} a_{DIM_{kr}} Z_r(D) + \sum_{r=0}^{3} p_{DIM_{kr}} Z_r(D) + \sum_{s=0}^{1,2} a_{T_{ks}} Z_s(T) + \sum_{s=0}^{1,2} p_{T_{ks}} Z_s(T) + eijkl\_DT,$$

where,

y_{ijkl_DT} = observation for a given trait at a certain DIM = D, and, temperature TAVE or TMAX) = T

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HYS_i = herd-year-season of test day; seasons defined from January to March, April to June, July to September and October to December (3,899 levels for milk and SCS, 3,898 levels in fat and 3,897 levels in protein)

ADIM_j = age-DIM (130 levels)

b_r = regression coefficient for temperature

$Z_r(T)$ = Covariate of the rth Legendre coefficient evaluated at temperature = T

$a_{\text{DIM}_{kr}}, p_{\text{DIM}_{kr}}$ = additive genetic and permanent environmental random regression coefficients for DIM for animal k (r=0,3), respectively,

$Z_r(D)$ = Covariate of the rth Legendre coefficient evaluated at DIM=D

$a_{T_{kr}}, p_{T_{kr}}$ = additive genetic and permanent environmental random regression coefficients for T for animal k (alternatively, r=0,1 or r=0,2), respectively,

e_{ijkl_DT} = residual effect, with e_{ijkl} i.i.d. $N(0, \sigma_e^2)$

The (co)variance structures for regression coefficients for individual animals for both the regressions on DIM and temperature were assumed to be:

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

where, $\mathbf{a}$ and $\mathbf{p}$ are vectors of additive genetic and permanent environmental coefficients for all animals, respectively, and, $\mathbf{G_o}$ and $\mathbf{P_o}$ are the additive genetic and permanent environmental (co)variances for all the regression coefficients , respectively. Dimension of $\mathbf{G_o}$ and $\mathbf{P_o}$ matrices varied depending on the degree of the polynomial fitted to the temperature coefficients and included non-zero covariances between coefficients associated to DIM and to the temperature.

Overall, four models were fitted for each trait-temperature combination. Models differed in the number of coefficients fitted for the polynomial functions for additve genetic and permanent environmental effects for DIM and temperatures as covariates. First, models including regressions for temperature only and varying the degree of the polynomial fitted from quadratic (TEM2) to cubic (TEM3) were solved. Then models

fitting cubic random regressions on DIM together with a quadratic (DIM3TEM2) or, alternatively, a cubic (DIM3TEM3) polynomial regression on temperature were analyzed.

The additive genetic deviation for individual animals at time t and the additive and permanent environmental variances at time t were computed from the estimated solutions for genetic regression coefficients and the estimated (co)variance matrices as in Jamrozik and Schaeffer (1997). Genetic parameters (heritability and correlations) at different time points were obtained from the estimated (co)variances.

A Bayesian approach via Gibbs sampling to obtain samples from marginal posterior distributions of the parameters of interest was used using a software written in fortran90 language (López-Romero *et al*., 2003).

Statistical model comparison

The described models were compared according to the goodness of fit, using the logarithm of the marginal density (LMD) and to the predictive ability of future data, using a checking function that measures the expected difference under the predictive distribution (D) between an observation excluded from the total data to fit the model and its prediction. Both statistics are calculated by the program within the Gibbs sampling. Details of the calculation procedure for the model comparison statistics can be found in López-Romero et al. (2003). The best model is the one with the minimum value of D or with the maximum value of LMD.

Post-Gibbs analyses were performed using the boa package of R (R3.2 version 2.15.2). Convergence of Gibbs chains was monitored by visual inspections of plots of samples.

3. Results and Discussion

3.1. General description of the final data

Production data

Table 3.1 presents summary statistics of phenotypic data for the final data used in the subsequent analyses. Data correspond to 128,112 Spanish Holstein cows calving between 2002-2012 and 2,514,762 test-day records for five lactations. The observed means for analyzed traits are generally coincident with results from other Holstein populations found in literature (Aguilar *et al*., 2009; Bastin *et al*., 2012; Bohmanova *et al*., 2008; Hammami *et al*., 2013; Smith *et al*., 2013).

Table 3.1. Mean, standard deviation (SD), percentiles 1 and 99 (in brackets), and animal and data number for the analyzed traits.

	Mean	SD	Percentiles [1%-99%]	Animals number	Data number
Milk yield (kg/d)	30.7	8.98	[11.20-52.10]	128,112	2,514,762
Fat (%)	3.56	0.78	[1.77-5.46]	128,003	2,466,055
Protein (%)	3.27	0.34	[2.59-4.20]	127,977	2,475,092
Fat yield (kg/d)	1.07	0.34	[0.39-2.04]	128,003	2,466,055
Protein yield (kg/d)	0.99	0.26	[0.39-1.60]	127,977	2,475,092
SCC[1]* (thousands/ml)	275	549.71	[8-2979]	127,831	2,473,022

* SCC = Somatic cell count

Figure 3.1 presents the average phenotypic level of the analyzed traits per lactation and number of control. Daily production was lower for the first eight controls of first lactation and more persistent in this lactation when compared with the rest. Peak production was observed at the second control (between 33 and 95 days in milk) at around 30 vs. 39 kg for first vs. second and later lactations. Due to the large differences in milk production, tolerance to heat stress has been found to be higher during first lactation (Aguilar *et al*., 2009). Moreover, Aguilar *et al*. (2009) found that the largest negative correlation between general production level and heat tolerance differed between lactations and stage of lactation, being more negative at 50 DIM and 200 DIM for the first and later parities, respectively. Milk production stayed at relatively high levels (nearly 20 kg/d) at the 14th control, showing a justification for extension of the previously standard 305 d of lactation length. Percentage of fat and protein and SCC

followed a reverse pattern from that of milk yield due to a dilution effect. Increasing values of SCC were observed in successive lactations.
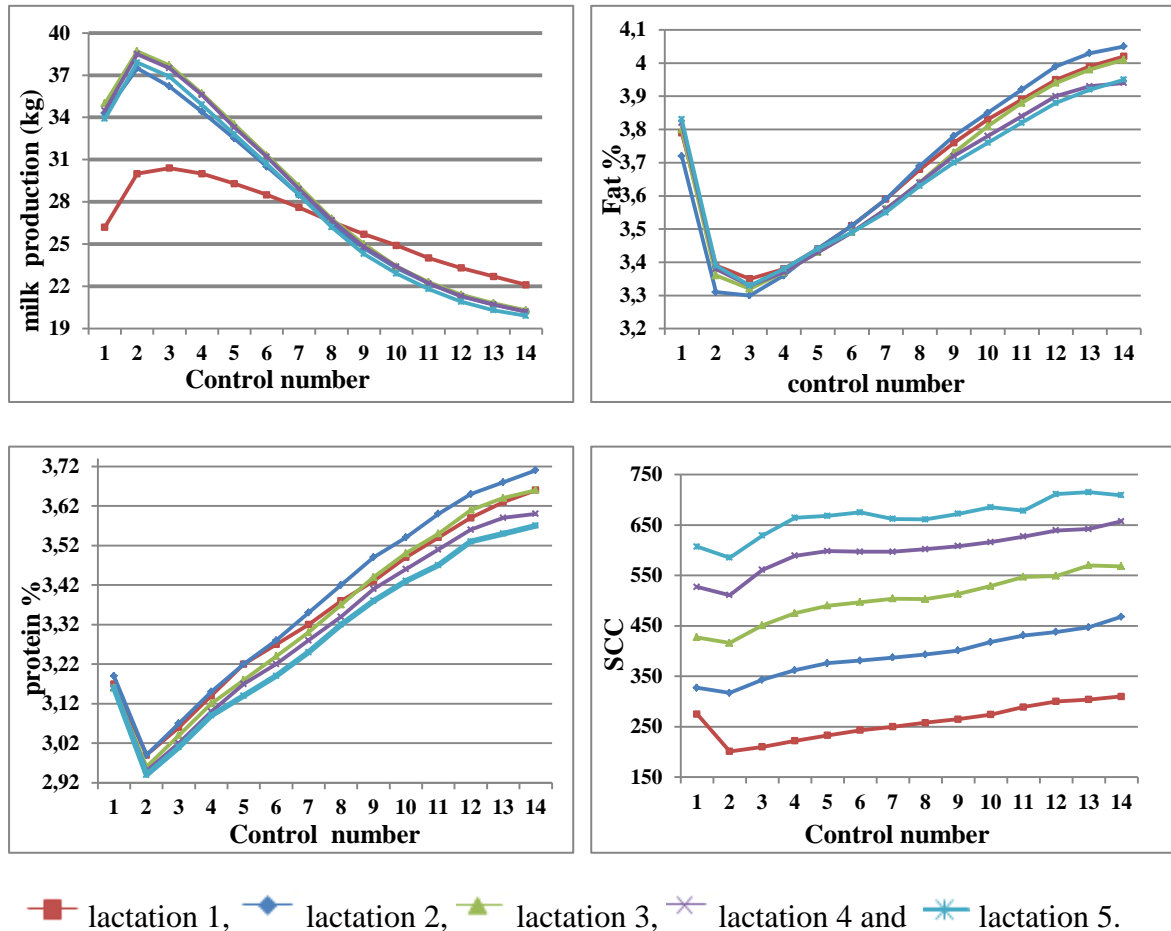
Figure 3.1. Average of daily milk production, fat and protein percentage (%) and somatic cell count ($\times$ 1000 cells /ml, SCC) per lactation and control number.

Graphics in Figure 1 show the large effect of days in milk and control number in the values of the analyzed traits. Values for daily milk production can double when peak and last controls are compared for later than first lactations and SCC can be three times as high in fifth vs. first lactation. This is important to take into account when looking at patterns of raw production with climatic variables if the distribution of DIM is not even.

Meteorological parameters:

Figure 3.2 shows the variation throughout the year of average values of THI per maximum temperature (TMAX) and average temperature (TAVE). Maximum daily average temperature and THI attained 26.5 ºC and 73.5 respectively in the month of

July. Daily maximum temperature and THI were recorded in the same month at 34.5ºC and 88.3, respectively. The minimum temperature and THI values recorded for both temperatures (TAVE and TMAX) and THI (average and maximum) were 8.0ºC, 12.9ºC, 47.0 and 56.0, respectively, detected in January.
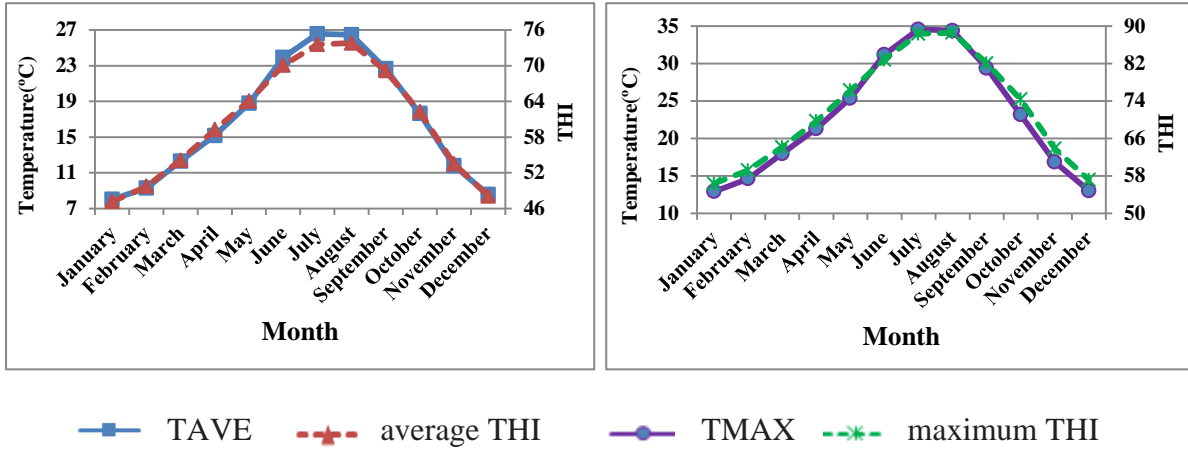
Figure 3.2. Variation through the year of average (left) and maximum (right) values of THI and temperature.

According to Figure 3.2, the curves of temperature and THI are almost overlapped for both average and maximum weather parameters. Therefore, the temperature data were used as weather variable for later analyses because of the high correlation between temperature and THI curves and because of the relatively large amount of missing data of humidity in Andalucía. Carabaño *et al*. (2013) showed that the models with average temperature only adjusted better than models with THI in two small ruminant populations. Moreover, Bohmanova *et al*. (2007) found that humidity was the limiting factor of heat stress in humid climates, whereas dry bulb temperature was the limiting factor of heat stress in dry climates, such as the studied regions.

Meteorological stations and farms distance

Figure 3.3 shows a histogram for distances from farms to the closest meteorological station with either temperature or THI for each test day of recording. The average distance between weather stations and farms for average temperature was 7.24 km with a maximum of 29.65 km and a standard deviation of 5.0, whereas the average distance for the THI was 13.29 km, with a maximum of 29.98 and a standard deviation of 7.90. The larger distance observed for the THI adds another argument in

favour of the use of temperature as weather parameter to measure heat stress. The figures for distances found in this study are within the values found in the literature. Hammami *et al.* (2013) reports as mean, maximum, and minimum distances between weather station and herd location of 7.6, 16.0, and 1.0 km, respectively, Menéndez-Buxadera *et al.* (2011) an average distance under 25km and Zumbach *et al.*, (2008) a distance of 14.0 km and 21.0 km from the two farms in the study. This Figure also shows that average distance by temperature lower than average temperature by THI (7.24 km) thus better accuracy and other raison to use model with temperature only.
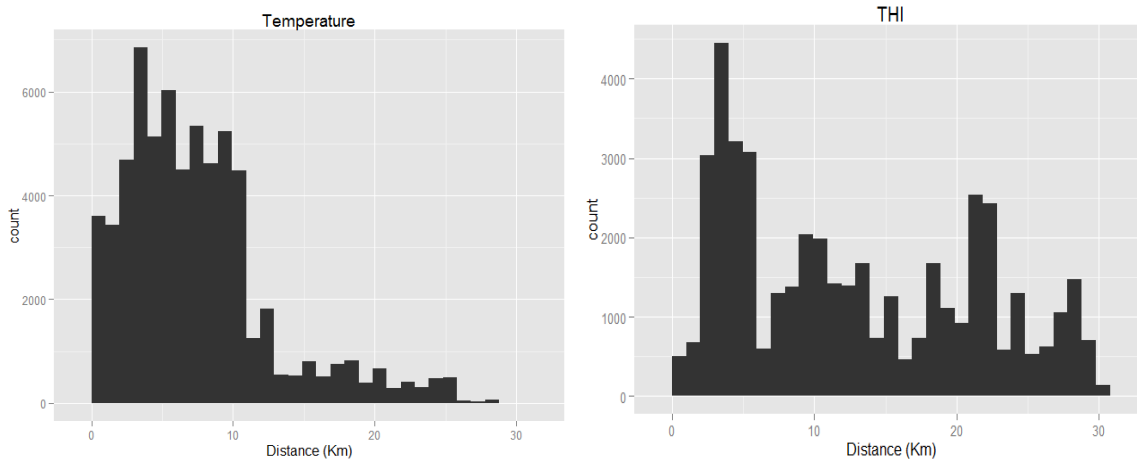
Figure 3.3. Histogram of distances from farms to meteorological stations providing average daily temperature and average daily temperature and relative humidity (THI) for each test day of recording.

Date of weather recording

Coefficients obtained in the ridge regression of production and SCC on the day of recoding and the previous 15 days are shown in Figure 3.4. This analysis had the objective to identify the date of weather recording that has a larger influence on the recorded traits in a certain test day. In other words, the day associated with the coefficient showing the most negative values for production and positive values for SCC.
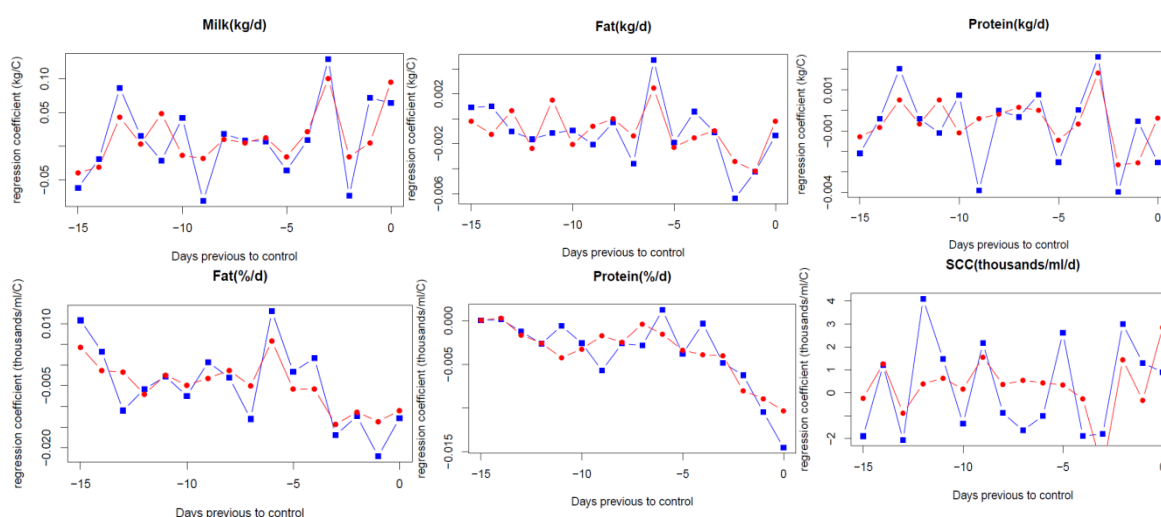
Figure 3.4. Estimates of regression coefficients on average (TAVE=blue line and squares) and maximum (TMAX= red line and circles) daily temperature at test day (0) and one to 15 days earlier obtained in the ridge regression analyses for the production traits and SCS.

According to these curves, only kg and percentages of fat and protein followed a more clear pattern of more negative regression coefficients as the date of temperature recording approaches the test day. Milk yield and somatic cell production showed a quite random pattern. Thus, the temperature on the test-day was used in the following analyses. In other side, other authors (Bohmanova *et al*., 2007; Brügemann *et al*., 2011 and Hammami *et al*., 2013) used the THI of three days previous to control for their studies.

3.2.Reaction norm of production to temperature increase. Population level

The aim of this study was to determine the comfort region for temperatures and the subsequent decay in production or increase in SCS. The splines models provide estimates of two parameters, the threshold and slope of decline in production and they have a clear biological interpretation. In SP two splines are fitted. The first corresponds to the comfort region, where production or SCS remain unaffected by temperature, and the second describes the stress region, where production and SCS follow a linear decay or increase with temperature. The point where the two splines meet is the thermotolerance threshold. On the other hand, polynomials provide estimates of regression coefficients, with a difficult biological interpretation. In order to provide

parameters with a biological meaning, maximum and derivatives of the polynomial functions were calculated per LP models. In order to provide a picture of the fitting process of the SP and LP functions for each trait-temperature combination, average and adjusted (by environmental and animal effects in [1]) production and SCS at successive temperature degrees together with the adjusted LP and SP functions are shown in Figure 3.5. Adjustment by environmental effects had a large impact on the observed pattern, particularly for milk and fat yield. Milk production showed a cold stress region after data adjustment as well as a heat stress area. Fat yields tended to decrease in all the range of temperatures for the raw data and this yielded low threshold values for the SP models. The pattern of SCS to increasing temperatures for both raw and adjusted records was quite erratic.
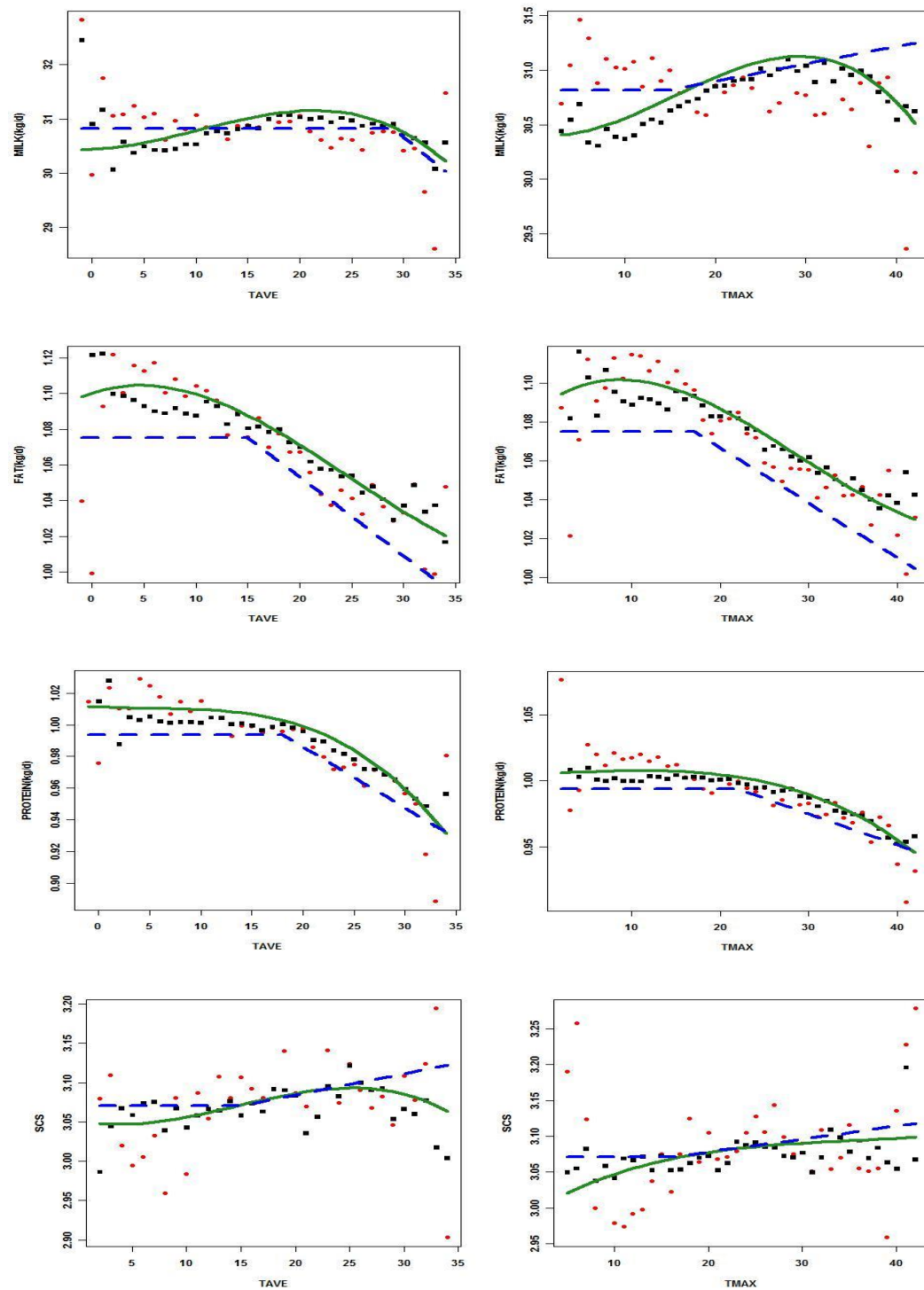
Figure 3.5. Average of raw (red dots) and adjusted by environmental and animal effects (black squares) records by degree of average (TAVE) and maximum (TMAX) daily temperature and polynomial (green solid line) and splines (blue discontinuous line) fits for production traits and SCS.

Tables 3.2 and 3.3 present the results of estimated tolerance thresholds and slopes for the SP models and the proxies for those, the maxima and derivatives of the polynomial function for the LP models.

Table 3.2. Posterior means and 95% high posterior density intervals (in brackets) of the threshold parameter in the spline model and maxima in the Legendre polynomial models.

	TAVE			TMAX		
	Threshold (ºC)	Slope (daily g/ºC)	Derivative at threshold (daily g/ºC)	Threshold (ºC)	Slope (daily g/ºC)	Derivative at threshold (daily g/ºC)
Milk	29.2	-157.4	-87.6	15.0	15.9	39.4
		[-203.4, -116.9]	[-94.2, -79.8]		[15.0, 16.9]	[37.5, 41.6]
Fat	15.0	- 4.4	-2.9	16.6	-2.8	-1.9
		[-4.5, -4.3]	[-3.0, -2.8]		[-2.9, -2.8]	[-2.0, -1.8]
Protein	18.1	-3.8	-1.6	21.5	-2.3	-0.9
		[-4.0, -3.7]	[-1.7, -1.6]		[-2.4, -2.3]	[-0.9, -0.8]

Table 3.2 showed the declined of production traits and increase in SCS by degree of TAVE and TMAX for SP and LP models. The daily losses in Table 3.7 can be used to determine the economic losses associated to heat stress such as in the study of St-Pierre et al. (2003). For milk production, a decline of -157.4 and -87.6 g/d/°C of TAVE above the threshold of thermotolerance was found by SP and LP models, respectively for TAVE. For TMAX, no relevant figures are provided given the fact that the comfort threshold estimated by the SP model was too low. Derivatives of the polynomial function at 30, 35 and 40 ºC of TMAX were -88.0, 800.2 and 1722.4 g/d/°C. For fat and protein yields, the estimated slope from SP models was larger than the derivative at the SP threshold, as might be expected from the gradual slopes of decrease provided by polynomial functions. A decrease of around -4.4 and -3.8 g/d/°C of TAVE were found for fat and protein yields, respectively, with the SP models. For TMAX the decay estimated for these traits was smaller, -2.8 and -2.3 g/d/°C, for fat and protein, respectively. For LP models, decay in fat and protein production was estimated as -66.9/-62.5 and -67.6/-105.9 g/d/°C at 25/30 ºC of TAVE, respectively. For TMAX, the declines were similar when maximum daily temperatures were 35/40ºC.

Goodness of fit of the models fitted to the studied traits were compared using the DIC values for this criterion are presented in Table 3.3 for SP and LP models with either TAVE or TMAX as measures of heat load..
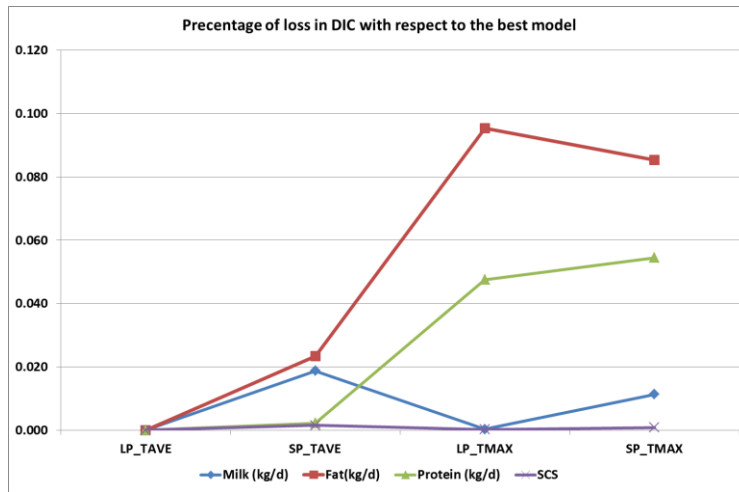
Table 3.3. Deviance information criterion (DIC) values for splines and polynomial models for production traits and somatic cell score (SCS) with average (TAVE) and maximum (TMAX) daily temperatures. Minimum (best) values for DIC are in boldface.

	TAVE		TMAX	
	Splines	Polynomial	Splines	Polynomial
Milk	6570261.7	**6569032.3**	6569773.7	6569053.0
Fat	-798892.8	**-799079.3**	-798397.4	-798317.3
Protein	-1709251.6	**-1709289.1**	-1708358.6	-1708476.4
SCS	3505640.7	**3505586.9**	3505615.3	3505594.0

For all traits, the LP model (a cubic polynomial) with TAVE as measure of heat load showed the best fit of the data. Polynomials are more flexible functions and can take into account gradual changes in the slope of reaction of the traits to incresing temperatures. Moreover, in the case of milk yield, a cold stress region was also detected, which cannot be accomodated with the SP model used in this study. Average temperatures might be thought of as better indicators of heat load than daily maxima because they may account for the fact that lower temperatures at night, which will lower the average, might alleviate the day high temperatures effect.

To compare model improvement across traits, relative values of the DIC were obtained. Relative values are expressed as percentage of the loss in goodness of fit between the model in question and the model with best (smallest) DIC within trait.

Results in Figure 3.2 indicate that losses in goodness of fit were different for milk vs. fat and protein yields. SCS showed nearly no effect of the heat stress function fitted or of the heat load variable used. For milk yield, larger losses were observed when fitting SP vs. LP models while the use of TAVE vs. TMAX did not change the goodness of fit within a model. On the contrary, the use of TAVE vs. TMAX had a larger impact on goodness of fit for fat and protein than the type of model used, SP vs. LP.

Figure 3.6. Percentage of loss in the Deviance Information Criterion (DIC) with respect to the best model in each trait, for splines (SP) or Legendre polynomials of third degree (LP) fitted for productive traits and somatic cell score (SCC) on daily average (TAVE) or maximum (TMAX) temperature in the test day.

3.3. Reaction norm of production to temperature increase. Individual genetic and environmental components

Estimated average curves of response to temperature

Estimated curves for each trait temperature combination under the four models analyzed are presented in Figure 3.7. The effect of temperature on milk yield was very small and showed an upward trend with temperature. The fact that the records analyzed come from animals in first lactation, with lower production levels might explain the lack of heat stress observed. For fat and protein, a decreasing trend was observed for all the trajectory when quadratic polynomials were fitted, while cubic polynomials allowed fitting cold and heat stress areas for fat production. As for the whole population study, low thresholds of change in slope were observed for fat and protein. SCS showed very little variation with increasing temperature. Inclusion of individual regressions on DIM did not modify the pattern of respond for the same degree fitted to temperature.
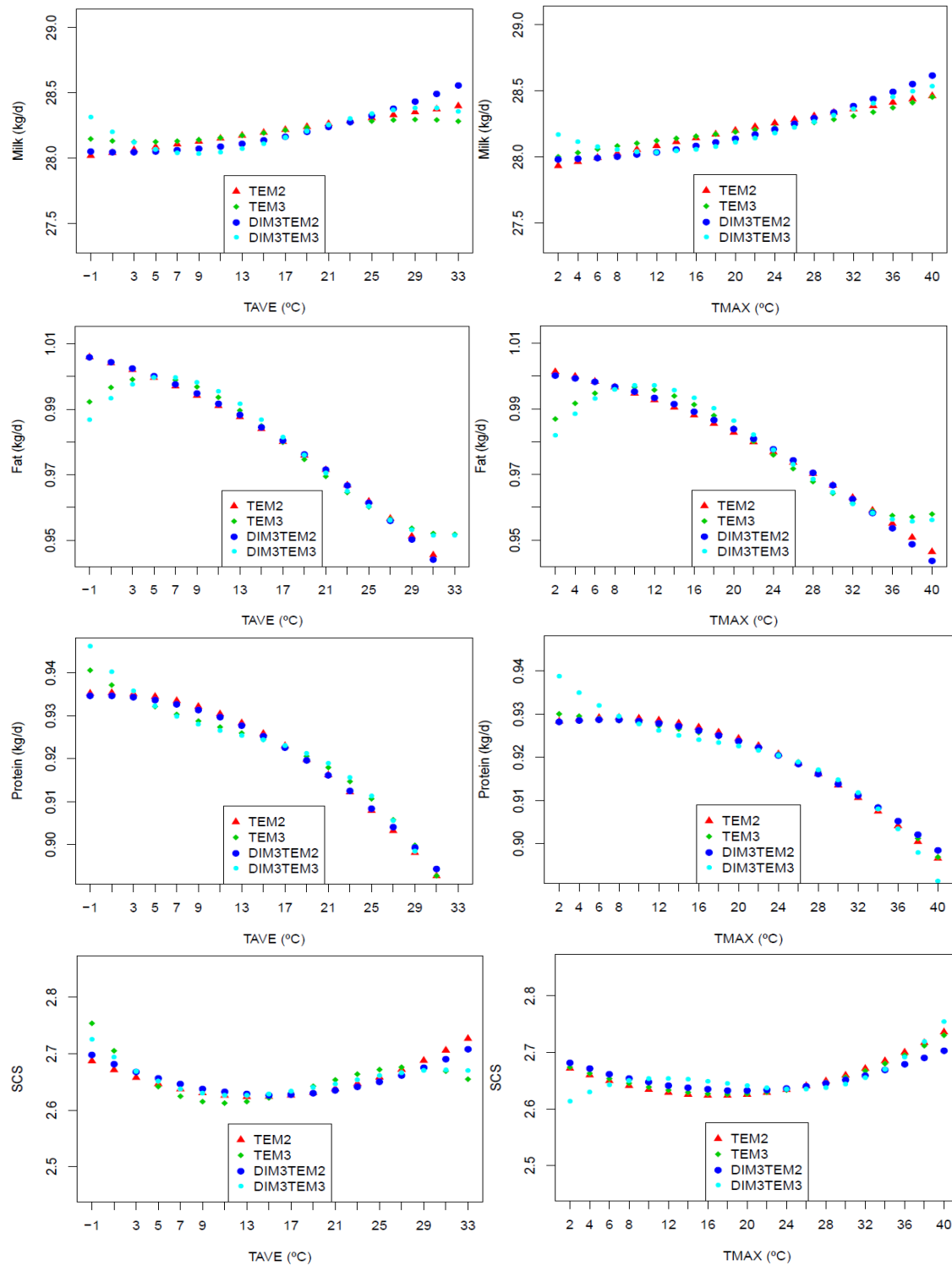
**Figure 3.7.** Mean curves of response obtained from the estimated regression coefficients for models fitting only quadratic (TEM2) or cubic (TEM3) individual polynomial regressions on average (TAVE) or maximum (TMAX) or models fitting individual cubic regressions for days in milk and quadratic (DIM3TEM2) or cubic (DIM3TEM3) polynomial regressions on temperature for production traits and somatic cell score (SCS).
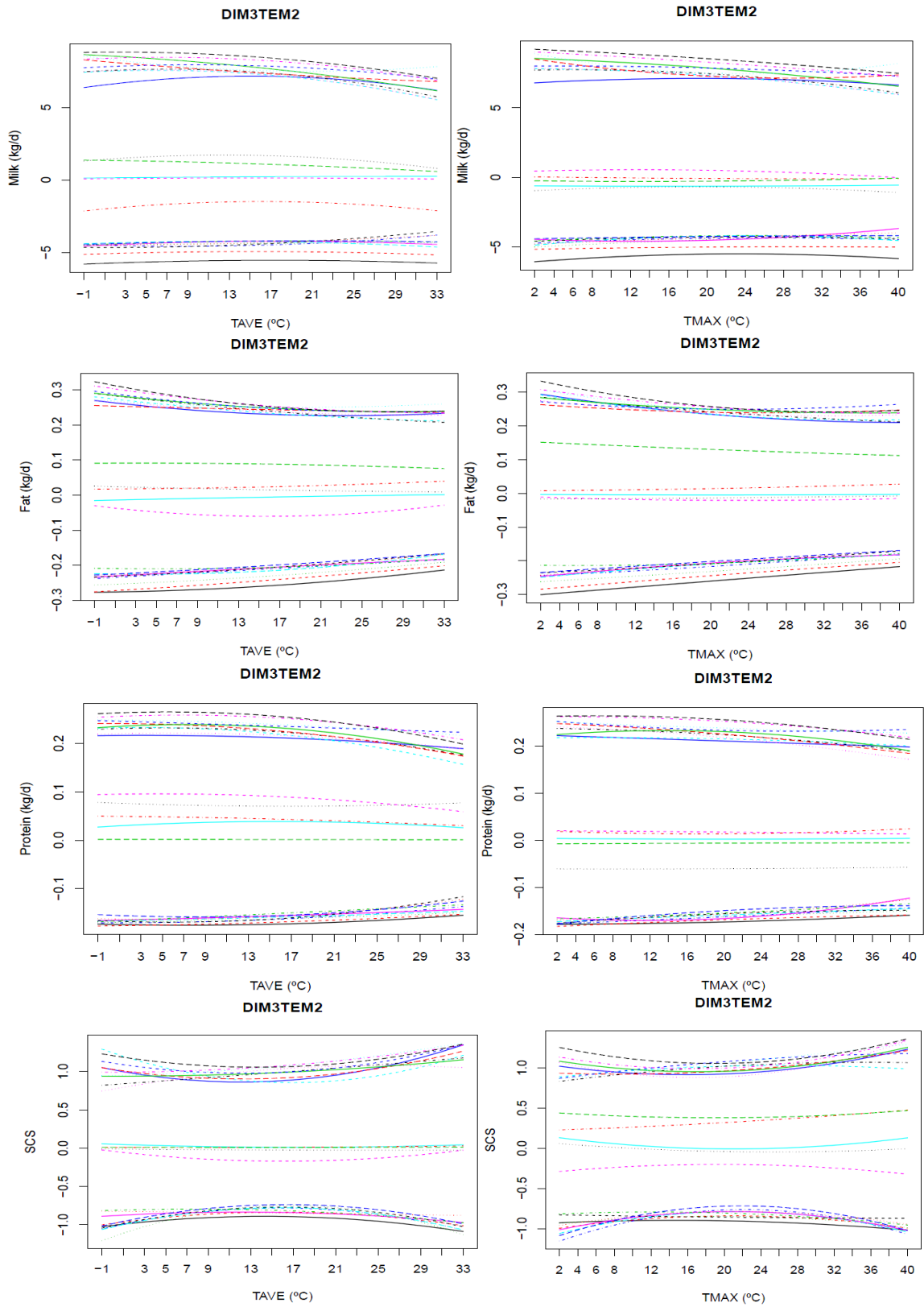
Estimation of breeding values

Figures 3.8 to 3.11 show estimated breeding values (EBV) for the four models analyzed for each combination of trait and temperature for the 10 top, 10 bottom and five random animals for the EBV of the estimated intercept under each model. These animals showed different types of response to increasing TAVE and TMAX values. Animals with high breeding value for production, a trend could be observed such that EBV decreased with increasing heat stress in terms of increasing daily temperature. For SCS the opposite trend was observed. For milk and SCS the trend was less notorious. In goats, Menéndez-Buxadera *et al*. (2012) categorized three types of animals, (i) Robust animals, which show a stable performance throughout the THI trajectory (with an average intercept and an average slope); (ii) Tolerant animals, which show a low genetic level (low intercept) and a high genetic capability to adapt to climatic stress (positive slope); and (iii) Non-tolerant animals, which manifest a high genetic level (high intercept) and very low capability to adapt to stressful climate conditions (negative slope). In our study, the animals with high genetic level were the ones most affected by temperature increasing (negative slope), whereas, the animals with a low genetic level for fat and protein did not show influence of temperature increasing. On the contrary, increases of estimated breeding values (positive slope) were observed and the animals with average EBVs did not change when temperature increased. These trends were observed for all models analyzed. Some reranking of animals was observed along the temperature range. This would indicate that sensitivity to increasing heat loads is different across animals and more notorious in highly producing animals. In other words, genetic variability associated to tolerance /susceptibility to heat stress has been detected mainly in the high producers. This result would indicate that the increase in metabolic heat associated to higher production might be one of the major components of heat susceptibility in Holstein dairy cattle.
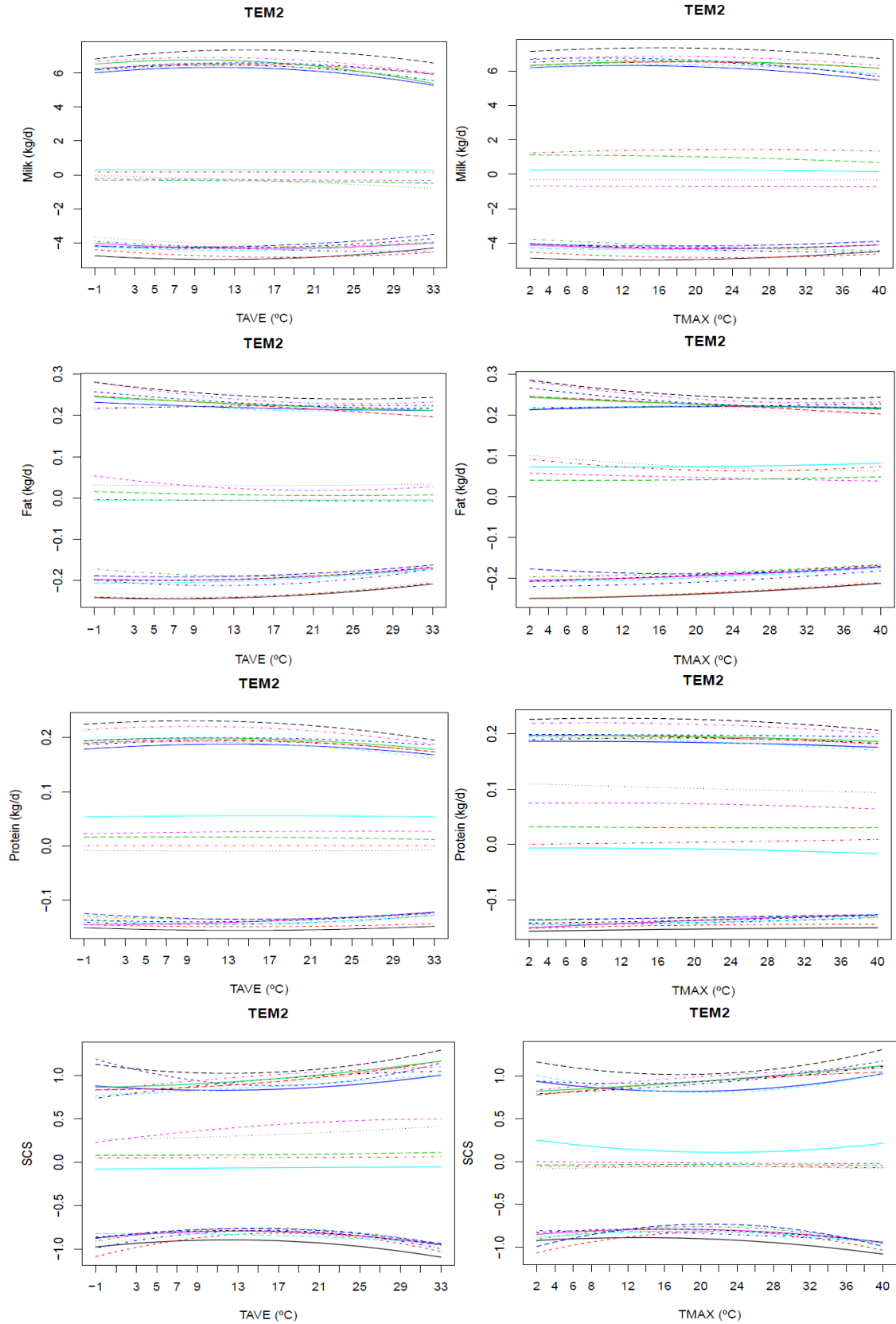
Figure 3.8 . Estimates of the breeding values for production traits and SCS by TAVE and TMAX and TEM2 model.
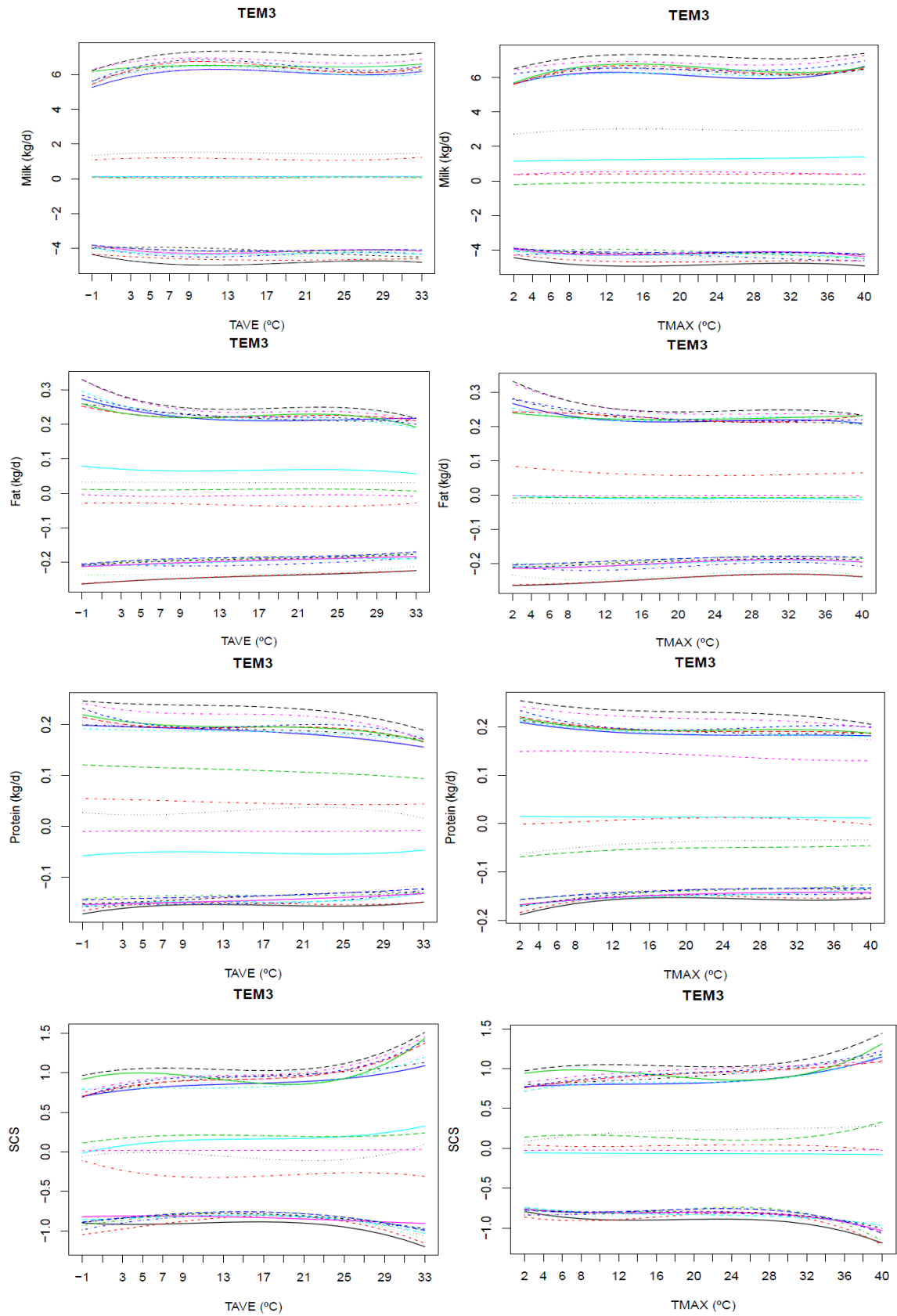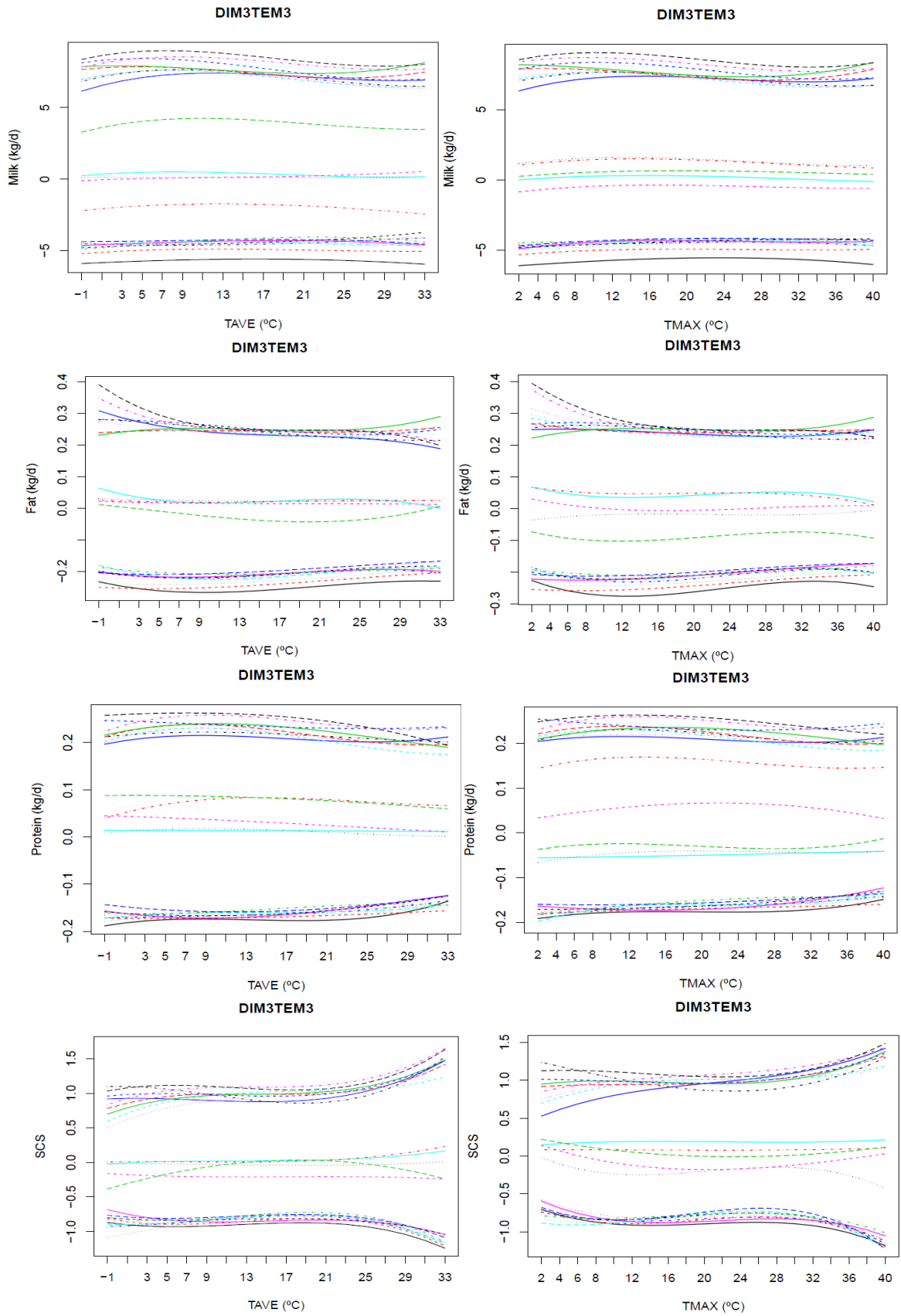
Figure 3.9 . Estimates of the breeding values for production traits and SCS by TAVE and TMAX and TEM3 model.
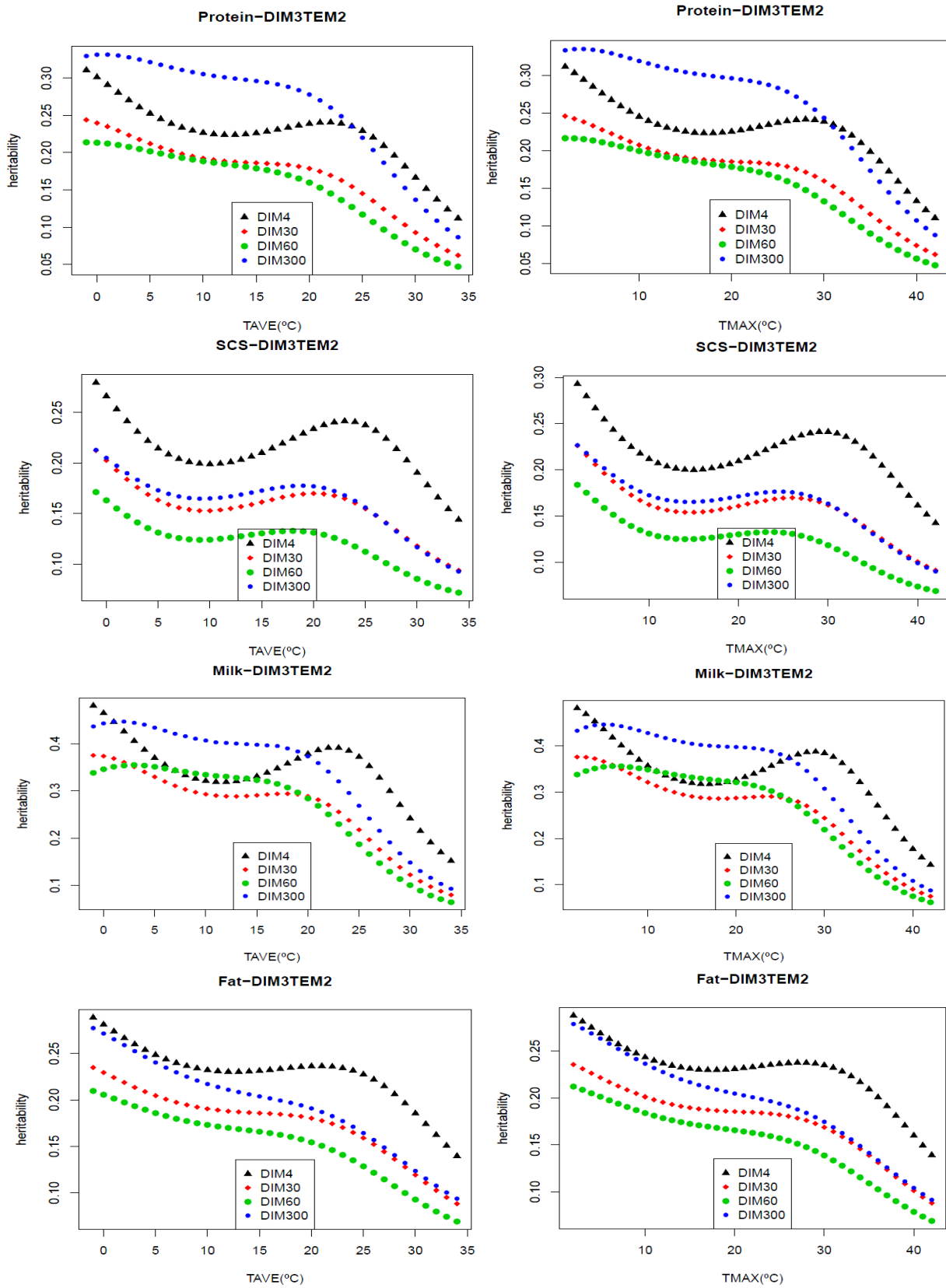
Figure 3.10. Estimates of the breeding values for production traits and SCS by TAVE and TMAX and DIM3TEM2 model.

Figure 3.11. Estimates of the breeding values for production traits and SCS by TAVE and TMAX and DIM3TEM3 model.

Figures 3.12 and 3.13 show curves of heritability estimates across temperatures for different lactation stages, early lactation (4 DIM), peak (30, 60 DIM) and end of lactation (300 DIM), within a given model. Only models fitting individual regressions on DIM, DIM3TEM2 and DIM3TEM3, could provide these curves. Heritability estimates were larger for milk yields (0.08-0.40) than for fat and protein (0.05-0.30) and lowest for SCS (0.05-0.25), being within estimates for these traits found in the literature. Heritability estimates tended to decrease at higher temperatures, being the slope of decay more steep after around 20-25ºC for TAVE and after around 30ºC for TMAX. For late stages, the threshold for the steeper decay was observed at higher temperatures than for the beginning and peak of lactation. Also, heritabilities tended to be lower for peak and early lactation than for the last stage of the lactation. Therefore, for temperatures at the extreme of the high range, heritability for production and SCS at the end of the lactation tended to be noticeably higher than for traits measured at the beginning or at the lactation peak. Thus, as for the breeding values, a relationship between the level of production (higher at DIM 4, 30 and 60 than at DIM300) and the effect of heat load was observed. Aguilar *et al* (2009) and Sanchez *et al*. (2009), in two studies on the US Holstein population in Georgia using a SP model found a negative estimated correlation between general and heat tolerance (negative of the slope) genetic components, indicating that animals with a high level of production are expected to be less tolerant, which is in agreement with the results observed in our study. Moreover, Aguilar *et al*. (2009) found that additive genetic effects for heat stress and yield traits increased greatly from the first to third parity. Consequently, later parity cows are expected to be much more susceptible to heat stress than are first-parity cows. In a German Holstein population of first lactation animals, Brügemann *et al*. (2011) under a polynomial random regression model similar to the one used in our study also found a decrease of heritability with increasing heat loads (THI in their case) and larger heritabilities for later lactation stages.
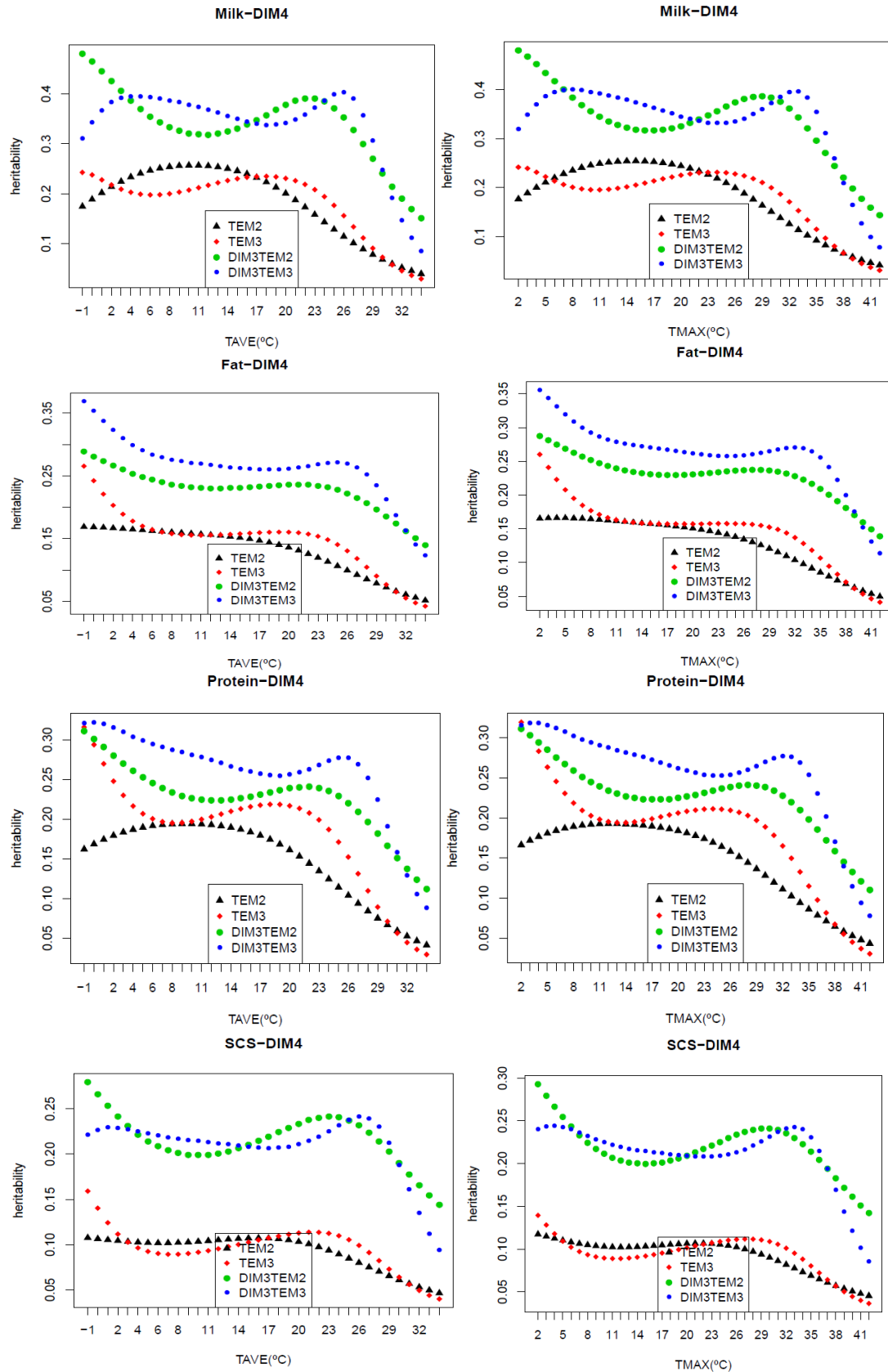
Figure 3.12. Heritability for production traits and SCS by TAVE and TMAX and by DIM3TEM2 model at different DIM.
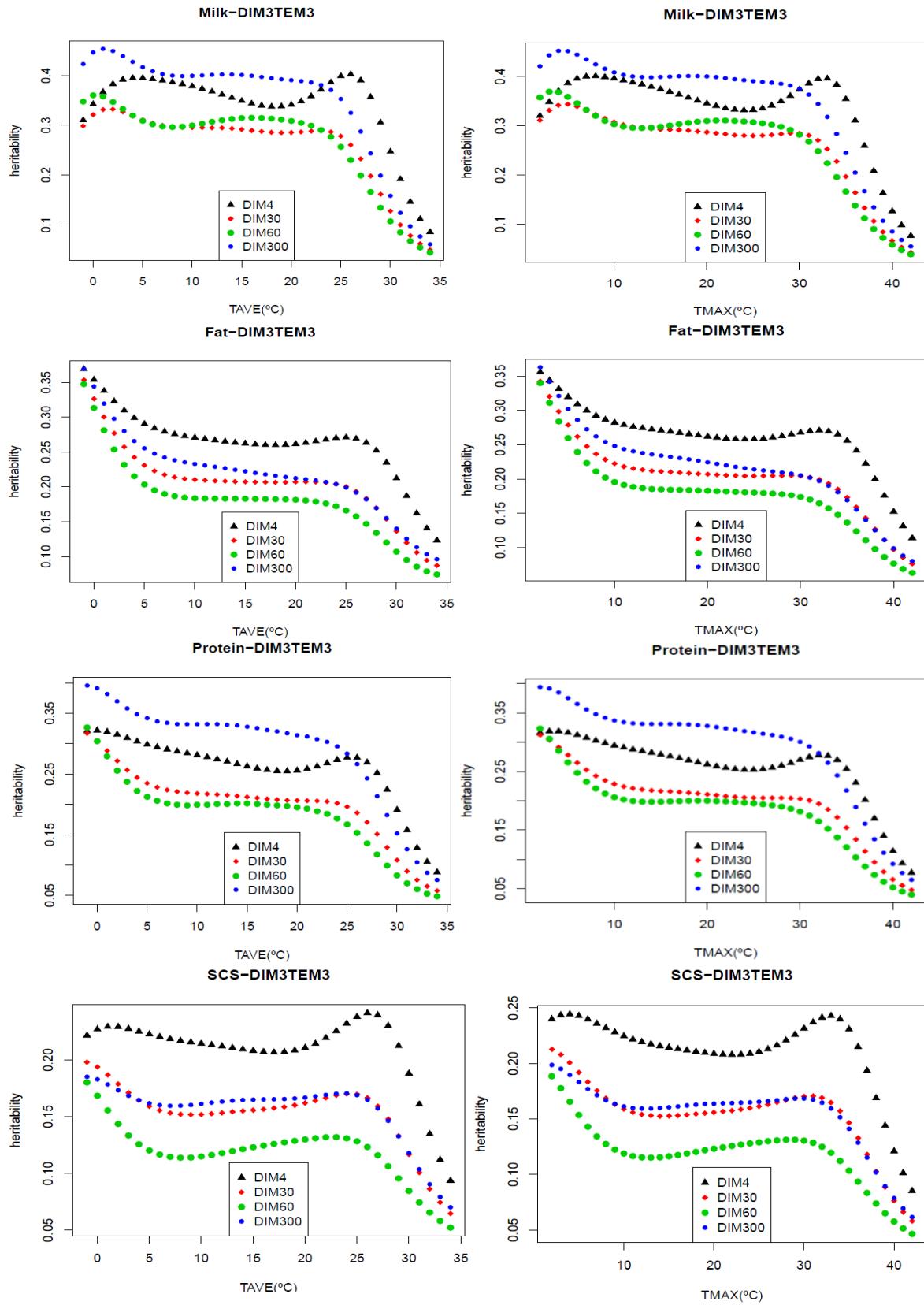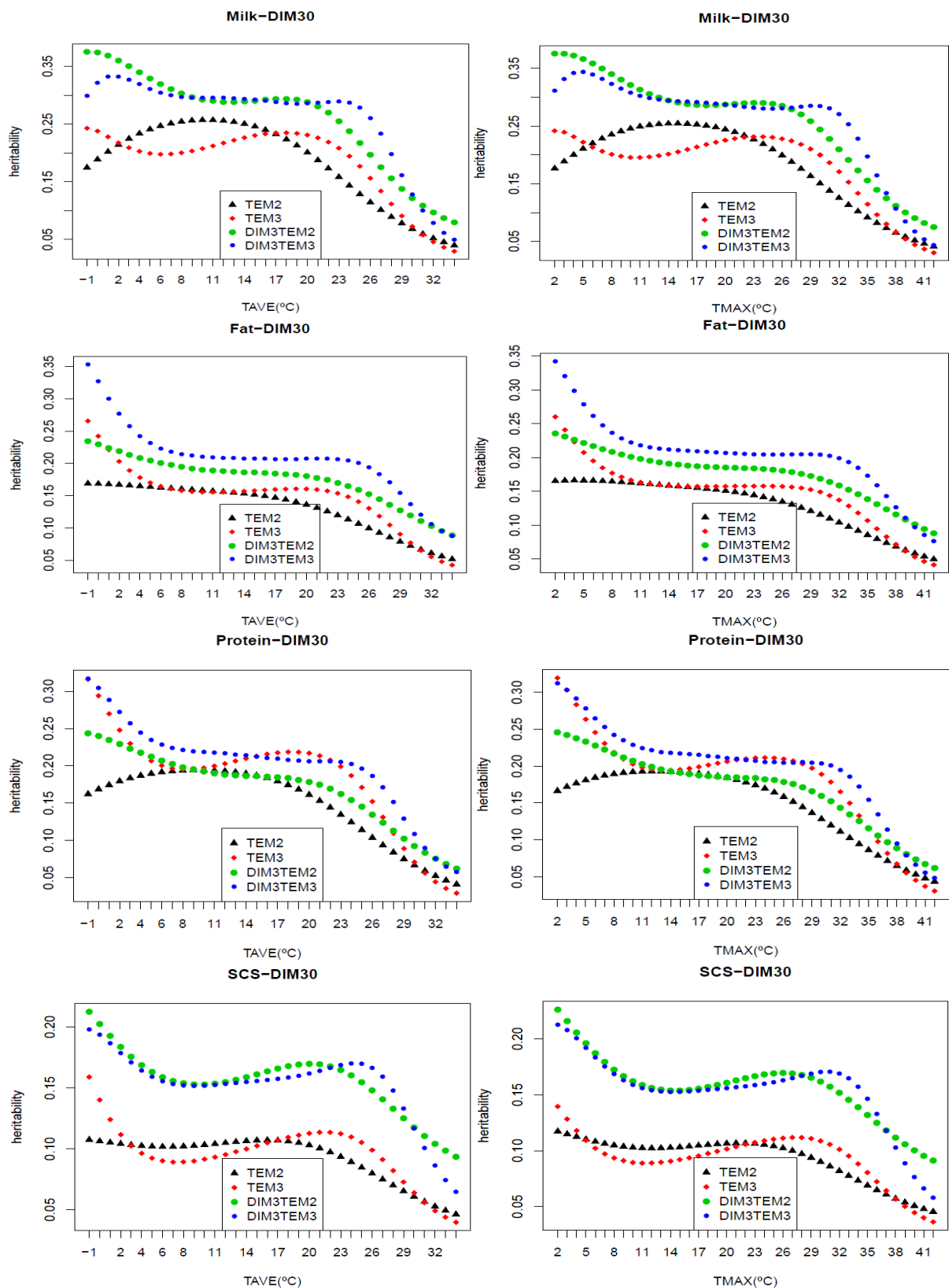
Figure 3.13. Heritability for production traits and SCS by TAVE and TMAX and by DIM3TEM3 model at different DIM.

Patterns of changes in heritability estimates across models (TEM2, TEM3, DIM3TEM2 and DIM3TEM3) with increasing temperatures for production and SCS measured at different stages of lactation (4, 30, 60, 300 DIM) are shown in Figures 14 through 17. The pattern of decreasing heritability estimates as temperatures increase observed for DIM3TEM_ models is now also observed for models not including the individual regressions on DIM (models TEM2 and TEM3). For these models, lower heritability estimates were observed. This trend was clearer for the end of lactation stage (300 DIM) and for SCS and milk yield, the traits that seemed to show less sensitivity to increases in heat load according to Figure 3.7. For quadratic and cubic polynomials not much differences in heritability estimates were observed except for the lower end of the range of temperatures (the cold part).

Figure 3.14. Comparison of Heritability estimates for production traits and somatic cell score (SCS) by TAVE and TMAX and different models (TEM2, TEM3, DIM3TEM2 and DIM3TEM3) at DIM 4.

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Figure 3.15. Comparison of Heritability estimates for production traits and somatic cell score (SCS) by TAVE and TMAX and different models (TEM2, TEM3, DIM3TEM2 and DIM3TEM3) at DIM 30.
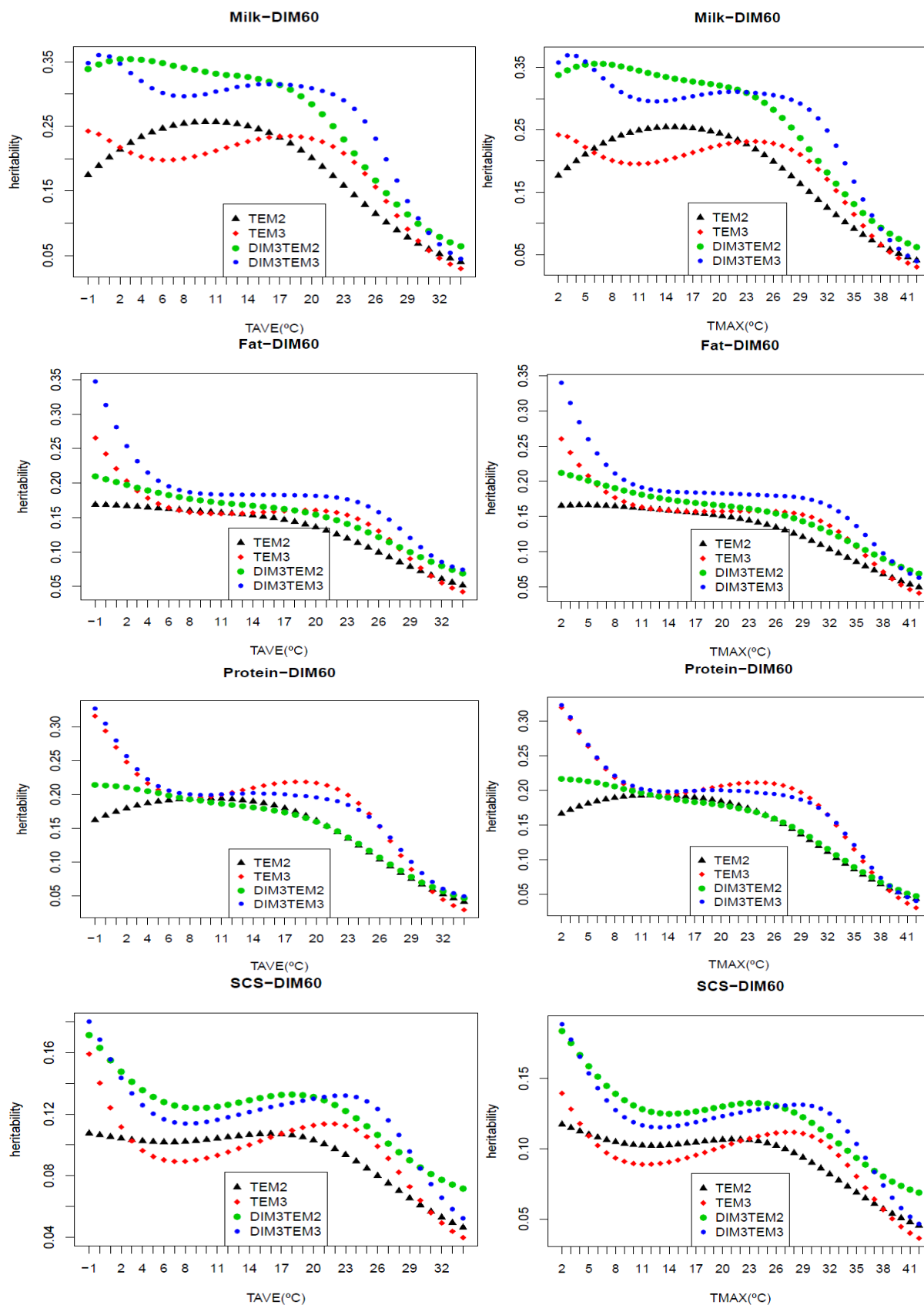
Figure 3.16. Comparison of Heritability estimates for production traits and somatic cell score (SCS) by TAVE and TMAX and different models (TEM2, TEM3, DIM3TEM2 and DIM3TEM3) at DIM 60.
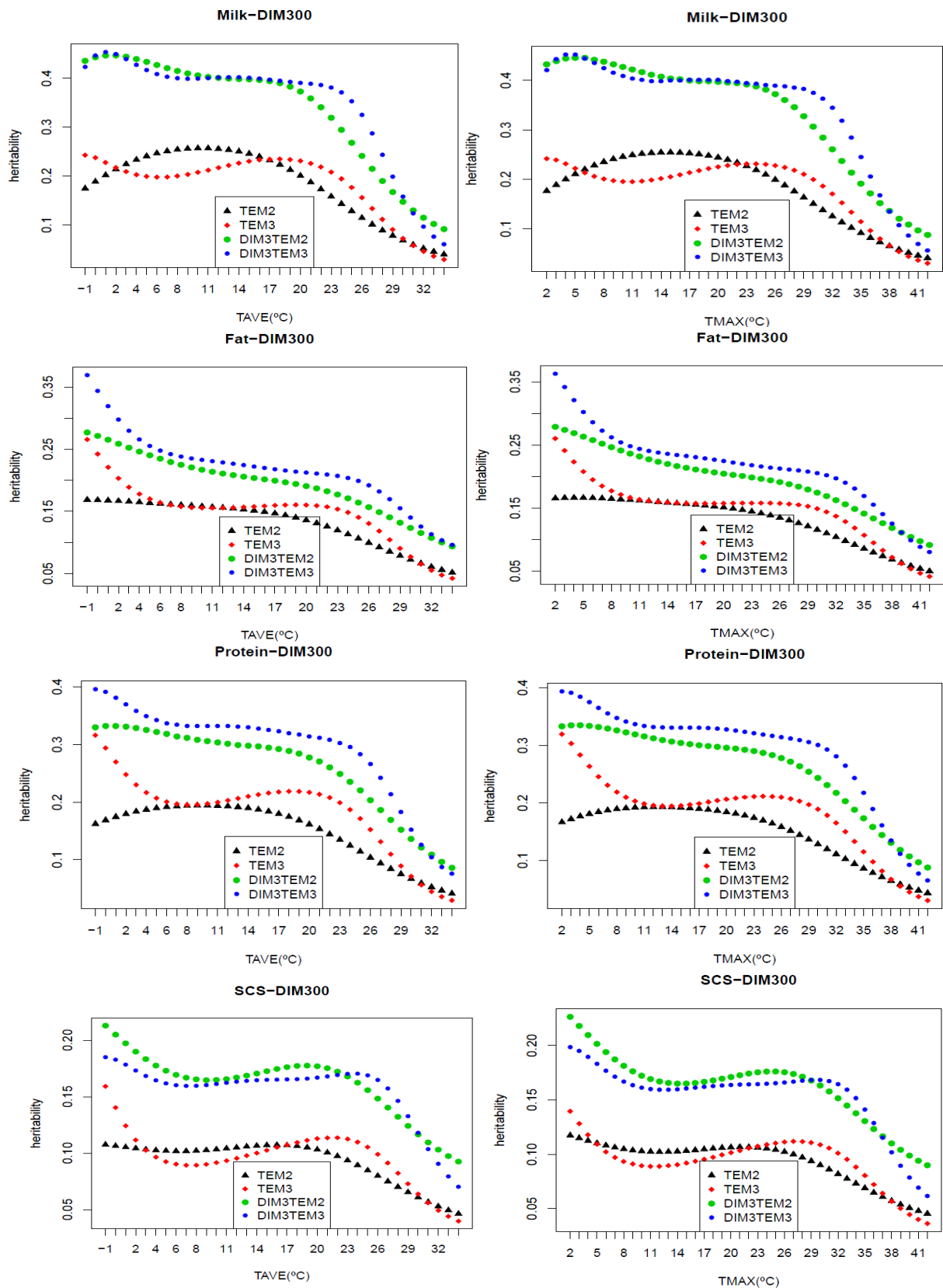
Figure 3.17. Comparison of Heritability estimates for production traits and somatic cell score (SCS) by TAVE and TMAX and different models (TEM2, TEM3, DIM3TEM2 and DIM3TEM3) at DIM 300.
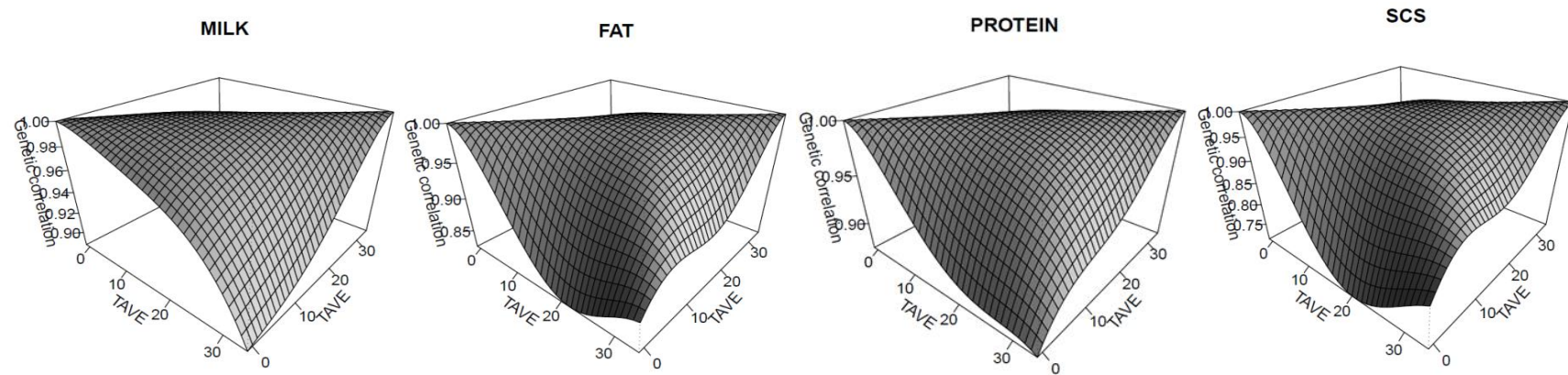
Genetic correlations for combinations of temperatures

Figures 3.18 and 3.19 show the estimates of the genetic correlations for combinations of temperatures. Estimates for genetic correlations from the model with Legendre polynomials of second and third degree among specific values for both daily temperatures (TAVE and TMAX) were >0.90 for milk, >0.70 for fat and protein and >0.50 for SCS. However, the estimates for genetic correlations obtained for protein by polynomials model of second degree and average temperature was >0.90, similar to results reported by Brügemann *et al.* (2011). These authors found higher genetic correlations between the additive genetic components at low and high values of the THI than the correlations found in our study for genetic values at the two extremes of the temperatures range. This was probably due to the less extreme temperatures probably found in Germany than in the Southern regions of Spain.
According to Robertson (1959) a genetic correlation below 0.80 is considered as an indication of the existence of an important effect of G×E. Therefore, GxE interaction might be considered to exist for fat and protein and SCS.

As expected, genetic correlations between adjacent temperatures were higher than for those for more distant temperatures. This was also reported by Brügemann *et al.* (2011).

a) genetic correlations for quadratic polynomial model

b) genetic correlations for cubic polynomial model
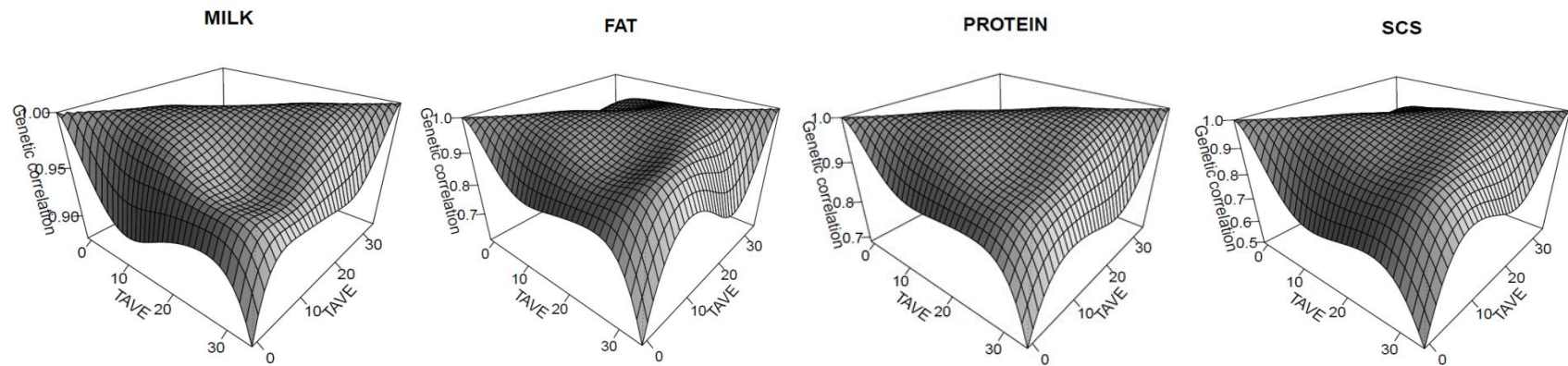
Figure 3.18. Genetic correlations for production traits and SCS between all combinations of average daily temperature (TAVE) and two polynomial models.

c) genetic correlations for quadratic polynomial model

d) genetic correlations for cubic polynomial model

Figure 3.19. Genetic correlations for production traits and SCS between all combinations of maximum daily temperature (TMAX) and two polynomial models.

Comparison of models:

Models used in this study were compared in terms of goodness of fit through the estimated log of the marginal distribution and the predictive ability as described in the M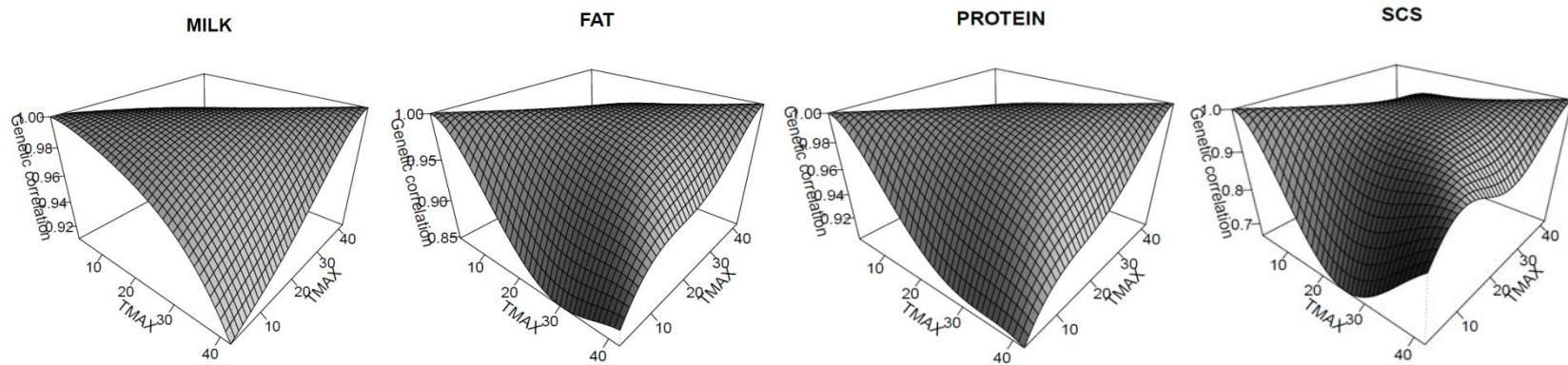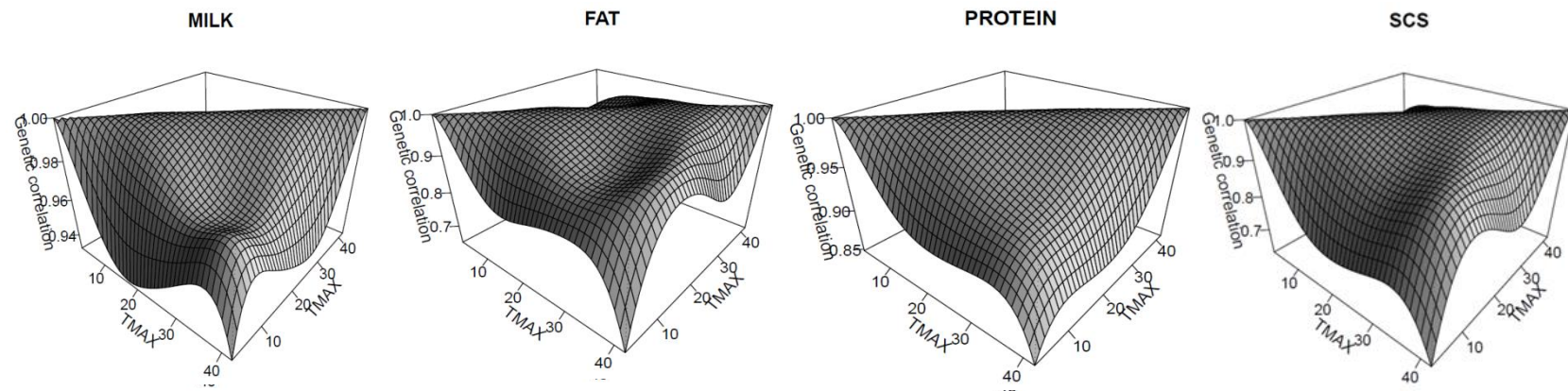aterial and methods section. Figure 20 shows the comparison of models adjusting using the marginal density, LMD, and the predictive ability parameter, D, in terms of improvement with respect to the worst model for each trait. Results for a reference model fitting only cubic polynomial regressions and no temperature effect are presented as well.
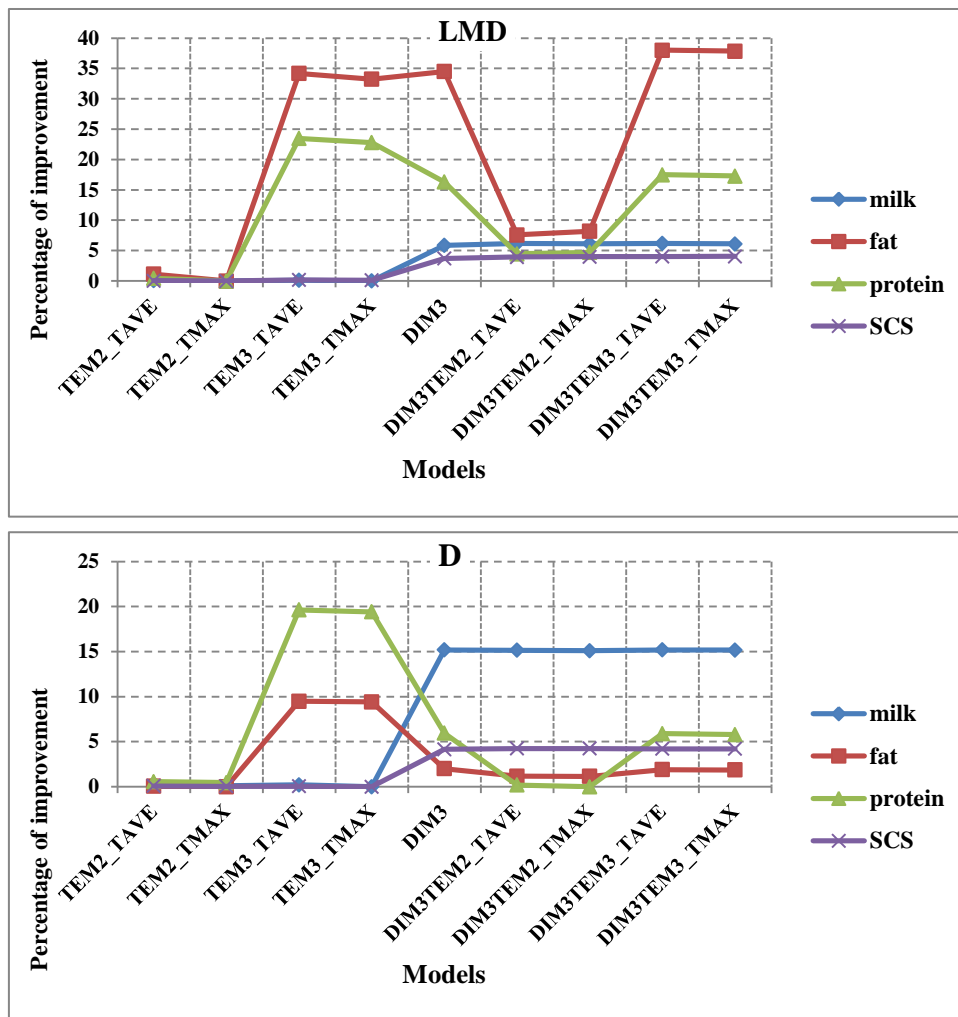
Figure 3.20. Percentage of improvement with respect to the worst model for each trait for the four polynomial models analyzed (TEM2, TEM3, DIM3TEM2 and DIM3TEM3) together with the reference model including only cubic regressions on days in milk (DIM3) on average (TAVE) and maximum temperature (TMAX) according to the logarithm of the marginal density of the data (LMD) and the predictive ability (D).

For both criteria, including regressions on DIM resulted in improved models for milk and SCS. This was more notorious for milk yield and the predictive ability criterion, which improved up to 15% when DIM regressions were included. On the other hand, addition of the temperature effect had nearly no effect in the goodness of fit or predictive ability of the model for these two traits. On the other hand, goodness of fit and predictive ability improved when a cubic polynomial on temperature was fitted to fat and protein yields. However, including regressions on DIM did not yield better models in terms of goodness of fit and even resulted in worse predictive ability. The discrepancy between the two criteria has been observed for overparametrized models that might show improved goodness of fit but worse ability of prediction of future records. Nearly no differences in the comparison criteria were found for TAVE vs. TMAX.

The improvement percentage of prediction ability of the model 4 was 38% more compared with the rest of the models. The worst improvement percentage of predictive ability detected by first model that was simple model with Legendre polynomials of second degree with temperature.

Pass from one model with Legendre polynomial of second degree for temperature to another model with Legendre polynomial of third degree for temperature greatly improves the model prediction with percentage of improvement 33 and 23% for fat and protein, respectively, greater relative of first model (1)

4. Conclusions

Conclusions

Increases in temperature have been associated to a decrease in production traits, mainly fat and protein production, and to a lower extent to increases in somatic cell scores. Heat tolerance thresholds have been found to be higher for milk (around 29.2ºC) and SCS than for fat and protein yields (around 18.1ºC).

Polynomial functions showed better convergence behaviour and provided better goodness of fit than the classical splines model used to model heat stress.

Genetic variability for response to increases in heat load has been observed. However, this variability may be mainly associated to level of production, so that animals with higher production levels seem to be more prone to suffer heat stress.

A cubic polynomial seems to provide better quality of adjustment than quadratic polynomial to determine both the population and individual deviations associated to increasing heat loads.

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