Effect of Heat Stress on Production Traits of Holstein Cattle in Japan: Parameter Estimation using Test-Day Records of First Parity and Genome Wide Markers

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Summary

Variance components were estimated for test-day milk (kg), fat (kg), protein (kg), and somatic cell score (SCS) using Gibbs sampling applying single step genomic BLUP (ssGBLUP). The phenotypes were 820,573 (752,514 for SCS) test-day records of 233 dairy farms in Japan with 1,170 randomly selected genotyped cows; the sample excluded Hokkaido (an island in the northern part of Japan where heat stress was minimal). Matrix H, which combined additive and genomic relationships, included 93,725 (86,435 for SCS) phenotyped cows and 8,401 genotyped bulls and cows. Dairy farms were linked to meteorological offices based on their covering areas for the announcement of weather forecasts, advisories, and warnings that reflect local climates, and then each phenotype was linked to the average temperature–humidity index (THI) for up to four days before each test day. Heat stress was defined as changes in phenotypes per unit increase in THI when THI increases were above the threshold of 60, and additive genetic (AG) and permanent environmental (PE) effects of the heat tolerance of each cow were added to the Japanese national genetic evaluation model. PE variances of heat tolerance were larger than AG variances of heat tolerance in all four traits. These results suggest that accumulation of various non-AG factors may affect the heat tolerance of individual cows. Average AG correlations between general effect and heat tolerance were negative, except for SCS. Therefore, antagonistic characteristics of the two AG effects should be carefully considered. With appropriate determination of THI, the use of total AG effects could be a feasible option. Inclusion of later parity is required for further study, as they are more affected by heat stress than the first parity. However, genetic evaluation of heat tolerance would be feasible.

Keywords: estimation of variance components, heat stress, Holstein, ssGBLUP

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Introduction

The impact of heat stress on dairy cattle production is not negligible. It can affect not only the profitability of each dairy farm but also the overall supply of dairy products to consumers. It is recommended that dairy farms take appropriate measures for heat stress management, such as using cooling fans. In addition, there is interest in the genetic improvement of heat tolerance, particularly in the Southern part of Japan. Such interest could increase in future due to the gradual and ongoing global climate change. However, the relevant studies are limited, and no study has used genotype information in Japan.

The objectives of this study is to estimate variance components for test-day milk, fat and protein yield (kg), and somatic cell score (SCS). We used Gibbs sampling applying single step genomic BLUP (ssGBLUP), including two random linear regressions describing the cows' heat tolerance.

Material and Methods

Phenotypic records

Test-day records of milk, fat and protein yield, (kg) and SCS (obtained from somatic cell count x (1000 cells / ml) by $\log_2(x/100) + 3$) from

purebred Holstein cows, collected in Japan other than Hokkaido (an island in the northern part of Japan where heat stress is minimal) between April 1987 and November 2015 were processed according to the data editing criteria of the Japanese national genetic evaluation. Then, records of 233 dairy farms with randomly selected genotyped cows were extracted for estimation of variance components. These farms were linked to meteorological offices based on their areas for the announcement of weather forecasts, advisories, and warnings that reflect local climates, and then each phenotype was linked to the average temperature–humidity index (THI) (NRC, 1971) for up to four days before each test day.

$$THI = (1.8 \times T_d + 32) - (0.55 - 0.0055 \times RH) \times (1.8 \times T_d - 26),$$

where T_d is dry bulb temperature in Celsius and RH is relative humidity in percentage.

Marker genotype

Genotypes of 5,439 bulls and 2,598 cows, obtained using Illumina BovineSNP50 BeadChips, versions 1 and 2 (Illumina Inc., San Diego, CA, USA), and genotypes of 20,411 cows, obtained using Illumina BovineLD BeadChips, were provided by the Holstein Association of Japan, Inc. Genotypes obtained by LD BeadChips were imputed using BEAGLE 3 (Browning and Browning, 2009), with the genotypes obtained by BovineSNP50 BeadChips used as reference. The call rates of animals exceeded 0.98. Markers on autosomes were selected according to the following criteria: call rate (>0.9), minor allele frequency (>0.01), and chi-square test p-value for Hardy–Weinberg equilibrium (>0.01), as described previously by Onogi *et al.* (2014). Consequently, the genotypes of 39,092 markers were available. To reduce equation size, the use of genotypes with LD chips in the statistical analysis was limited to those of cows with records and those of their dams. The records are summarized in Table 1.

Statistical analysis

A random regression test-day model was used in this study; the model was based on the national genetic evaluation as follows:

$$y_{ijklmno} = HTDT_i + M_j w + A_k w + hy_l v$$
$$+ pe_m z + peh_m \cdot f(THI) + u_m z + uh_m \cdot f(THI) + e_{ijklmno},$$

where $y_{ijklmno}$ = test-day yield in the contemporary group class i, comprising the head - test day - milking frequency, calving month j, calving age k of cow m belonging herd – calving year class l; $HTDT_i$ = the fixed effect of the contemporary group effect of class i; M_j = the row vector of fixed regression coefficients of calving month effect of class j; A_k = the row vector of fixed regression coefficients of calving age effect of class k; hy_l = the row vector of random regression coefficients of herd – calving year (HY) effect of class l; pe_m = the row vector of random regression coefficients of general permanent environmental (PE) effect of cow m; peh_m = the random linear regression coefficient of PE effect of heat tolerance of cow m; u_m = the row vector of random regression coefficients of general additive genetic (AG) effect of cow m; uh_m = the random linear regression coefficient of AG effect of heat tolerance of cow m; $e_{ijklmno}$ = heterogeneous random residuals corresponding to days in milk (DIM, t) categories n (t = 6–35, 36–65, 66–95, 96–125, 126–215, 216–305); $w' = \begin{bmatrix} \phi_0(t) & \phi_1(t) & \phi_2(t) & \phi_3(t) & \phi_4(t) & e^{-0.05t} \end{bmatrix}$, i.e., fourth order Legendre polynomials with a coefficient of the exponential term of the Wilmink function (Wilmink, 1987) at DIM t; $v' = \begin{bmatrix} \phi_0(t) & \phi_1(t) \end{bmatrix}$ i.e., linear Legendre polynomials at DIM t; $z' = \begin{bmatrix} \phi_0(t) & \phi_1(t) & \phi_2(t) \end{bmatrix}$ i.e., quadratic Legendre polynomials at DIM t;

$$f(THI) = \begin{cases} 0 \; if \, THI \leq 60 \\ THI - 60 \; if \, THI \geq 60 \end{cases}$$

i.e., the threshold of THI was set at 60, as by Nguyen *et al.* (2016).

Variance components were estimated using the "gibbs3f90" program of the BLUPF90 family (BGF90) that implements Gibbs sampling with a joint sampling of random correlated effects and traits (Misztal *et al.*, 2002). A single chain of 100,000 samples was run, with the first 50,000 samples discarded as burn-in. AG, PE, HY, and phenotypic variances and heritabilities were calculated for each combination of DIM and THI by using the remaining 50,000 samples. Convergence was determined by a visual inspection of the plotting of Gibbs samples.

To ensure the efficient use of all available phenotypic records and marker genotype data, we used ssGBLUP, which was based on the inverse of matrix *H* combining additive relationship and genomic relationship (Aguilar *et al.*, 2010, 2011). Default values of the program were used to construct *H*. We identified animals at least four generations from bulls with available phenotypic or genomic records.

Let $hy' = \begin{bmatrix} hy_1 & \dots & hy_l \end{bmatrix}$ be the overall vector of random HY effects; $pet' = \begin{bmatrix} pe_1' & peh_1' & \dots & pe_m' & peh_m' \end{bmatrix}$ be the overall vector of random PE effects; and $ut' = \begin{bmatrix} u_1' & uh_1' & \dots & u_m' & uh_m' \end{bmatrix}$ be the overall vector of random AG effects, the (co)variance structure was

$$\text{var} \begin{bmatrix} hy \\ pet \\ ut \\ e \end{bmatrix} = \begin{bmatrix} I \otimes Q & 0 & 0 & 0 \\ 0 & I \otimes P & 0 & 0 \\ 0 & 0 & H \otimes G & 0 \\ 0 & 0 & 0 & R \end{bmatrix},$$

where *I* is an identity matrix, *Q* is a 2×2 matrix of (co)variances for HY effects, *H* is a matrix combining additive relationship and genomic relationship, *P* and *G* are 4 (=3 regression coefficients of Legendre polynomials + 1 effect of heat tolerance) $\times$ 4 matrices of (co)variances for PE and AG effects, and *R* is a diagonal matrix with residual variance corresponding to DIM category l $\sigma_{e_l}^2$, respectively.

AG / PE effects and variances on test-day basis

The general and total AG effects of animal *m* at DIM *t* and specific THI were obtained as:

$$u_{m0}\phi_0(t) + u_{m1}\phi_1(t) + u_{m2}\phi_2(t); \text{ and}$$
$$u_{m0}\phi_0(t) + u_{m1}\phi_1(t) + u_{m2}\phi_2(t) + f(THI) \cdot uh_m.$$

General AG (co)variance at DIM *t* and *t'* was obtained by:

$$\begin{aligned} &\text{cov}(u(t), u(t')) \\ &= \text{cov}\left[u_{m0}\phi_0(t) + u_{m1}\phi_1(t) + u_{m2}\phi_2(t), u_{m0}\phi_0(t') + u_{m1}\phi_1(t') + u_{m2}\phi_2(t')\right] \\ &= \sum_{p,q} \text{cov}(u_{mp}\phi_p(t), u_{mq}\phi_q(t')) \\ &= \sum_{p,q} \phi_p(t)\phi_q(t')\text{cov}(u_{mp}, u_{mq}), \end{aligned}$$

where $\text{cov}(u_{mp}, u_{mq})$ is the *p*, *q* (=1, 2 or 3) element of *G*. AG variance of heat tolerance was obtained by $f(THI)^2 \cdot \sigma_{uh}^2$, where σ_{uh}^2 is a 4, 4 element of *G*.

AG covariance between general effect and heat tolerance at DIM *t* was obtained by:

$$\begin{aligned} &\text{cov}(u(t), f(THI) \cdot uh) \\ &= f(THI) \cdot \text{cov}(u(t), uh) \\ &= f(THI) \cdot \text{cov}\left[u_{m0}\phi_0(t) + u_{m1}\phi_1(t) + u_{m2}\phi_2(t), uh_m\right] \\ &= f(THI) \cdot \sum_i \phi_i(t)\text{cov}(u_{mi}, uh_m), \end{aligned}$$

where $\text{cov}(u_{ni}, uh_n)$ is the i, 4 element of *G*. AG correlation between general effect and heat tolerance was obtained by:

$$\frac{\sum_i \phi_i(t)\text{cov}(u_{mi}, uh_m)}{\sqrt{\sum_i \phi_i(t)^2 \text{cov}(u_{mi}, u_{mi}) \cdot \sigma_{uh}^2}}.$$

Finally, total AG variance was obtained by:
$$\sum_i \phi_i(t)^2 \text{cov}(u_{mi}, u_{mi}) + f(THI)^2 \cdot \sigma_{uh}^2 + 2f(THI) \cdot \sum_i \phi_i(t)\text{cov}(u_{mi}, uh_m).$$

General and total PE effect and variances may be obtained in a similar manner.

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Results and Discussion

The estimated AG variances of heat tolerance (σ^2_{uh}) and the PE variances of heat tolerance (σ^2_{peh}) are in Table 2. The fact that the PE variances of heat tolerance were larger than the AG variances of heat tolerance in all four traits suggests that accumulation of various non-AG factors may affect heat tolerance of individual cows.

The average AG and PE correlations between general effect and heat tolerance across lactation are presented in Table 3, and their changes are presented in Figure 1. The AG correlations were negative, except for SCS. Therefore, antagonistic characteristics of the two AG effects should be considered carefully for genetic improvement. In all four traits, the PE correlations were negative and weaker than the AG correlations.

The change of total AG and PE variances and heritabilities at specific THI are shown in Figures 2, 3, and 4. The higher the THI, the smaller the total AG variances became except for SCS. On the contrary, the higher the THI, the larger the total AG variances became for SCS partially due to opposite sign of genetic covariance between general effect and heat tolerance. Heritabilities of SCS were also larger for higher THI , whereas they were smaller in other traits across lactation.

The average of total AG and PE variances and heritabilities across lactation at THI = 60 / 80 are shown in Table 4. Similar trends in the previous Figures were observed except for average heritability of protein that was a little larger at THI = 80 than at THI = 60. AG and PE variances were smaller, than the first parity of the national genetic evaluation. As a result, heritabilities were also smaller. The inclusion of the effect of heat tolerance may be the reason for this difference; however, further studies are required.

Changes of the total AG variances were similar for milk and protein, but they were different for fat. Hammami *et al.* (2015) reported detailed study on changes in fat composition due to heat stress.   Such detailed study might clarify the difference further. This study did not use records of later parities that are affected by heat stress more than the first parity (Aguilar *et al.*, 2009). Inclusion of later parity is required for practical implementation.

Genetic evaluation of heat tolerance would be feasible. For practical implementation, the use of total AG effects with appropriate determination of THI could be an option as a solution to antagonistic characteristics of the two AG effects, rather than specific selection for heat tolerance.

Conclusion

Variance components were successfully estimated using ssGBLUP, with the model including effects of heat tolerance. Genetic evaluation of heat tolerance would be feasible; however, variance components, including later parities should be obtained for practical implementation.

Acknowledgments

References

Aguilar, I., Misztal, I., Johnson, D.L., Legarra, A., Tsuruta, S. & Lawlor, T.J. 2010. Hot topic: A unified approach to utilize phenotypic, full pedigree, and genomic information for genetic evaluation of Holstein final score. *J. Dairy Sci. 93,* 743–752.

Aguilar, I., Misztal, I., Legarra, A. & Tsuruta, S. 2011. Efficient computation of the genomic relationship matrix and other matrices used in single-step evaluation. *J. Anim. Breed. Genet. 128,* 422–428.

Aguilar, I., Misztal, I. & Tsuruta, S. 2009. Genetic components of heat stress for dairy cattle with multiple lactations. *J. Dairy Sci. 92,* 5702–5711.

Browning, B.L. & Browning, S.R. 2009. A unified approach to genotype imputation and haplotype-phase inference for large data sets of trios and unrelated individuals. *Am. J. Hum. Genet. 84,* 210–223.

Hammami, H., Vandenplas, J., Vanrobays, M.-L., Rekik, B., Bastin, C. & Gengler, N. 2015. Genetic analysis of heat stress effects on yield traits, udder health, and fatty acids of Walloon Holstein cows. *J. Dairy Sci. 98,* 4956–4968.

Misztal, I., Tsuruta, S., Strabel, T., Auvray, B., Druet, T. & Lee, D.H. 2002. BLUPF90 and related programs. In: *Proc. Seventh World. Congr. Genet. Appl. Livest. Prod.,* Montpellier, France. p. 1–2.

Nguyen, T.T.T., Bowman, P.J., Haile-Mariam, M., Pryce, J.E. & Hayes, B.J. 2016. Genomic selection for tolerance to heat stress in Australian dairy cattle. *J. Dairy Sci. 99,* 2849–2862.

NRC. 1971. A guide to environmental research on animals. Natll. Acad. Sci., Washington, DC.

Onogi, A., Ogino, A., Komatsu, T., Shoji, N., Shimizu, K., Kurogi, K., Yasumori, T., Togashi, K. & Iwata, H. 2014. Genomic prediction in Japanese Black cattle: Application of a single-step approach to beef cattle. *J. Anim. Sci. 92,* 1931–1938.

Wilmink, J.B.M. 1987. Adjustment of test-day milk, fat and protein yield for age, season and stage of lactation. *Livest. Prod. Sci. 16,* 335–348.

Table 1. Summary of records.

	Chip used for genotyping	Traits	
		Milk, Fat and Protein	SCS
Test-day records, n	-	820,573	752,514
Cows (female with records)	Total	93,725	86,435
	HD	807	
	LD[1]	363	
	-	92,555	85,265
Bulls (Sire of cows)	HD	3,126	
	-	2,229	
Females with genotypes but without records	HD	1,791	
	LD[1]	1	
Males other than bulls with genotypes	HD	2.313	
Other animals in a pedigree	-	106,843	101,777

[1]LD genotypes: only cows with records and their dams to reduce equation size.

Table 2. Estimated variances of heat tolerance

	Milk	Fat	Protein	SCS
AG (σ^2_{uh})	1.21×10^{-3}	1.66×10^{-6}	1.13×10^{-6}	3.15×10^{-5}
PE (σ^2_{peh})	6.51×10^{-3}	7.35×10^{-6}	5.52×10^{-6}	1.02×10^{-3}

Table 3. Average AG / PE correlations between general effect and heat tolerance across lactation.

	Milk	Fat	Protein	SCS
AG	−0.623	−0.564	−0.582	0.27
PE	−0.291	−0.388	−0.402	−0.124

Table 4. Average of total AG / PE variances and heritabilities across lactation.

		Milk	Fat	Protein	SCS
THI = 60	AG	4.41	5.63×10^{-3}	3.33×10^{-3}	0.108
	PE	7.03	9.76×10^{-3}	6.73×10^{-3}	0.652
	h^2	0.236	0.167	0.104	0.0643
THI = 80	AG	3.09	4.11×10^{-3}	2.37×10^{-3}	0.140
	PE	7.15	8.54×10^{-3}	5.86×10^{-3}	0.934
	h^2	0.177	0.133	0.134	0.0704
National genetic evaluation[1]	AG	6.84	9.28×10^{-3}	4.88×10^{-3}	-
	PE	9.01	1.35×10^{-2}	8.49×10^{-3}	-
	h^2	0.317	0.245	0.237	-

[1]The values of first parity. The national genetic evaluation did not use random regression model for SCS.
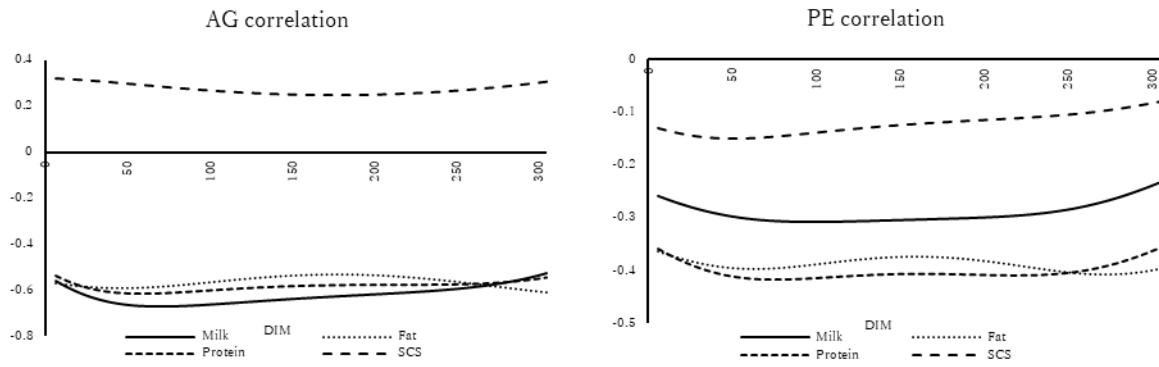
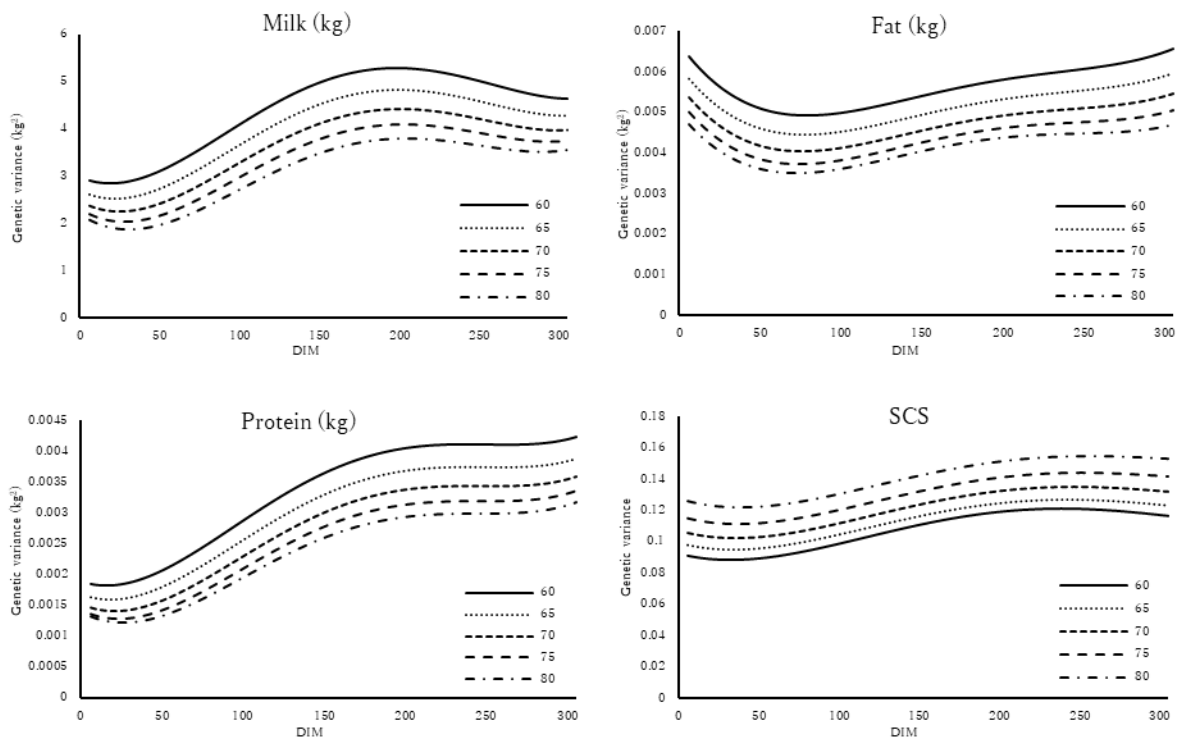**Figure 1.** AG / PE correlations between general effect and heat tolerance.
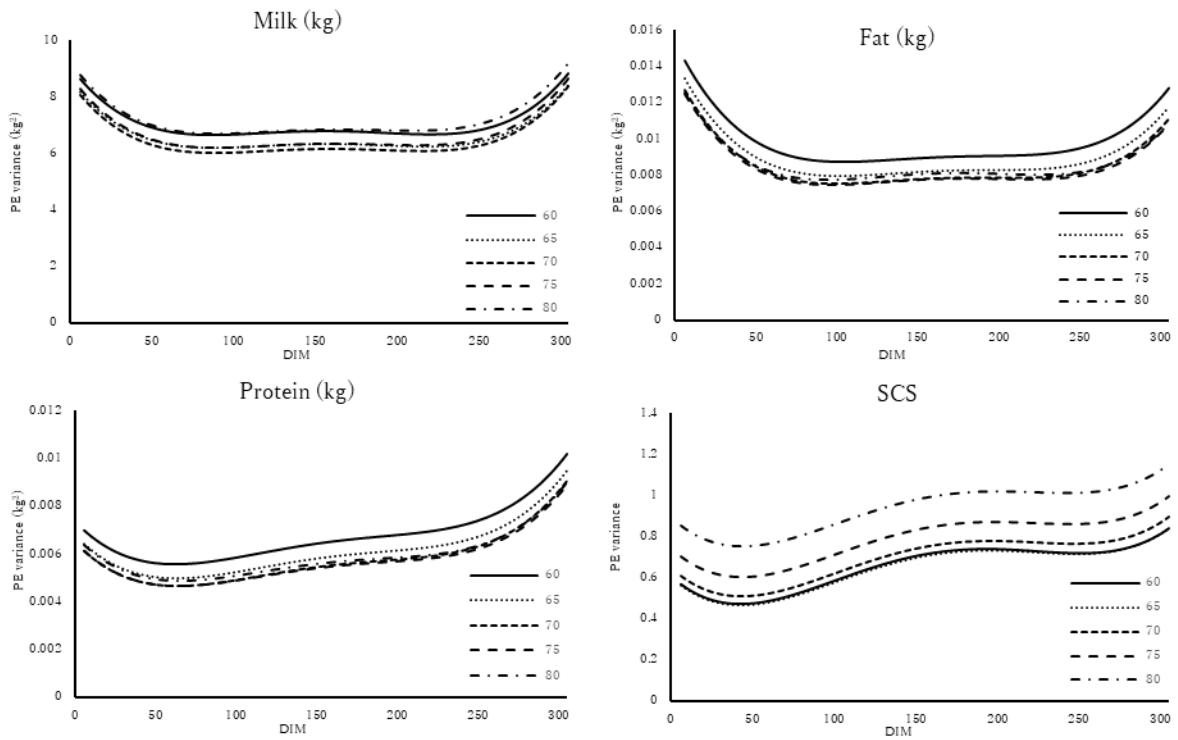

Figure 2. Total AG variances at specific THIs.
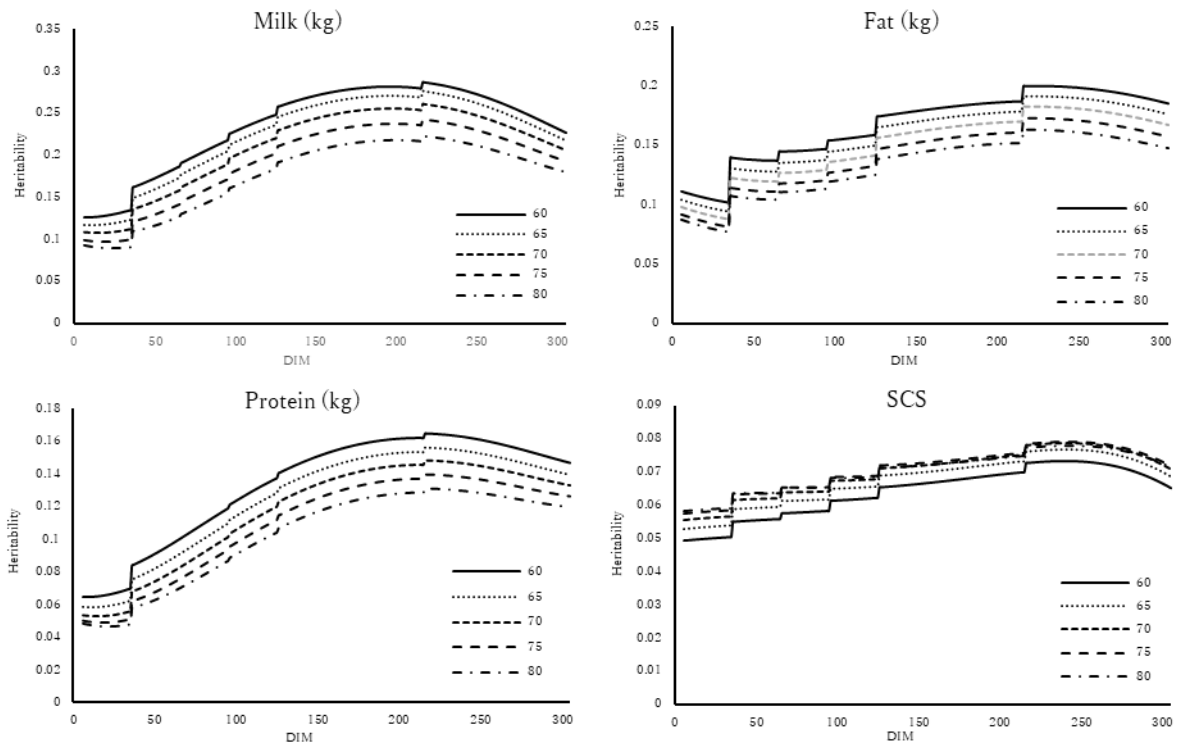
Figure 3. Total PE variances at specific THIs.

Figure 4. Heritabilities at specific THIs.