Lack of evidence for association between leptin/Sau3AI gene and milk yield traits in Holstein Friesian dairy cattle

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SUPPLEMENTARY FILE

Material and Methods

Experimental animals and data collection

The milk yield traits lactation milk yield (LMY; kg), activity (A; step/day), conductivity (C; m*S*/cm), milking time (MT; min), 305 days of lactation milk yield (LMY_{305}; kg), 200 days of lactation milk yield (LMY_{200}; kg), 100 days of lactation milk yield (LMY_{100}; kg), days in milk (DIM; day), peak milk yield (PMY; kg), days to peak milk yield (DPMY; day), average daily milk yield (ADMY; kg) and dry period (DP; day)) were collected from the herd management program of the enterprise. The activities of the animals during the day were determined with the help of the mobility measurement module and pedometers in the Afifarm herd management program (Ver 5.4.2). With the Afifarm MPC milk measure module, milk yield, milking duration and milk conductivity values were determined during milking of the animal with a deviation of less than 2%. In addition to all this information, numerical data such as parity and dry period of the animal were recorded were also recorded with Afifarm MPC. All obtained values were transmitted to the computer system via the online data line and transferred to excel from there. Also, LMY_{305}, LMY_{200}, LMY_{100}, PMY, DPMY and ADMY were obtained from the excel data. In the current farm, cows were milked three times in a day and artificially inseminated.

DNA extraction and PCR-RFLP method

Whole blood samples from each animal were taken in tubes containing Ethylenediaminetetraacetic acid (EDTA) from the Vena coccygea and stored at -18^{o}C until DNA extraction analysis. The EURX (Quick Blood DNA Purification Kit/Poland) commercial kit and the QuickGene Mini 80 (Medical Expo/USA) device were used to extract genomic DNA. In the study, primers (F 5’ -TGGAGTGGCTTGTTATTTTCTTCT 3’ and R 5’ -GTCCCCGCTTCTGGCTACCTAACT 3’F) discovered by Liefers *et al.* (2002) were used to determine the *Sau3A*I polymorphism in the intron 2 region of the leptin gene. The PCR reaction was performed in 20 µl reaction volume. The polymerase chain reaction (PCR) comprised of 4 µmol L^{-1} genomic DNA, 10 µmol L^{-1} 2x HOT FIREPol® Blend Master Mix

PCR Master Mix (2X; Solis BioDyne/Estonia), 0.5 µmol L^{-1} of each primer, and 5 µmol L^{-1} ddH_2O in a 20 µl volume. The amplification was performed in a gradient thermal cycler (T100 Thermal Cycler/Axonia Medical/Singapur) using the following program: an initial denaturation step at 94 °C for 2 min, followed by 35 cycles of 94 °C for 60 s, 58.1 °C for 60 s and 72 °C for 60 s. The final extension was at 72 °C for 15 min. 5 µmol L^{-1} PCR products were used for control and 15 µmol L^{-1} for cutting. The PCR products were digested with 0.5 U of *Sau3A*I restriction enzyme in 25 µl volume (Thermo Fisher Scientific). The restriction fragments were subjected to electrophoresis on 2% agarose with ethidium bromide gel in 1X TBE buffer and then visualized under UV light and scored in a gel documentation system.

Statistical analysis

The PopGene Version 1.32 software (Yeh *et al.*, 1997) was used for statistical analysis of allele and genotype frequencies and heterozygosity (Nei, 1973) of the gene region. The Chi-square (χ^2) test was performed to determine whether the population was in Hardy-Weinberg equilibrium (Düzgüneş *et al.*, 1983). Since the number of animals in the first lactation is high, the analysis of the data is not suitable for the repeated measurement of mixed model, therefore we used the General Linear Model to evaluate the effects of genotype and environmental factors are shown below. However, factors such as year, parity, and covariance vary in the models according to the different traits. Tukey's multiple comparison test was performed to assess the differences between means that were significant as a consequence of the analysis of variance. Minitab v16.1.1. software package (Minitab, 2010) was used for statistical analysis.

$Y_{ijklm} = \mu + \alpha_i + \beta_j + S_k + G_l + Cov_{(A,B)} + \varepsilon_{ijklm}$

Y_{ijklm}: observed traits in *ijklm*-th animal,

µ: mean of traits for population,

α_i: effect of years (*i* = years),

β_j: effect of seasons (*j* = seasons),

S_k: effect of parity (*k* = parity),

G_l: effect of genotype, (*l* = genotypes),

$Cov_{(A,B)}$: the estimated value of genetic or environmental covariance between two traits,

ε_{ijklm}: random error.

The Multiple Trait Derivative Free Restricted Maximum Likelihood (MTDFREML) package was utilized in the study to estimate variance components and genetic parameters using the Best Linear Unbiased Prediction (BLUP) method (Boldman *et al.*, 1995). The genotype was not included in the statistical model used to estimate variance components and heritability. Also, factors such as year, parity, and covariance vary in the models according to the different traits. In the estimation of variance components, heritability, and EBVs, the statistically significant factors (*$P<0.05$, **$P<0.01$) as a result of the analysis of variance were included in the model (Supplementary Table S1).

The following are the matrix descriptions of the models used to calculate the variance components (Mrode, 2014). The variance components were determined using Model, which included additive genetic effect (a) and maternal effect (m).

$Y = Xb + Za + Wm + e$

Y: A vector of the observation for each feature

X, Z ve W: matrices related to fixed and random factors

b: Fixed effects vector (such as parity, calving year, and season)

a: A vector of direct additive genetic effect

m: A vector of maternal effect

e: A vector of random error

The variance-covariance structure of Model was shown as a matrix below.

$$V \begin{vmatrix} \sigma_a^2 & \sigma_{am} \\ \sigma_{am} & \sigma_m^2 \end{vmatrix}$$

With this model, direct additive genetic effect (σ_a^2), genetic covariance between maternal effect and direct additive genetic effect (σ_a^2 x σ_m^2), maternal effect (σ_m^2), environmental variance (σ_e^2), phenotypic variance (σ_p^2), and the logarithm of likelihood (-2 Log L) of variance components were estimated. The variance components and heritability were estimated using the Restricted Maximum Likelihood (REML) technique in the MTDFREML program. Below are the models used to calculate the heritability of direct additive genetic effect (h_a^2) and maternal effect (h_m^2).

$h_a^2 = \sigma_a^2/(\sigma_a^2 + \sigma_m^2 + \sigma_a^2 \text{ x } \sigma_m^2 + \sigma_e^2) = \sigma_a^2/\sigma_p^2$

$h_m^2 = \sigma_m^2/(\sigma_a^2 + \sigma_m^2 + \sigma_a^2 \text{ x } \sigma_m^2 + \sigma_e^2) = \sigma_m^2/\sigma_p^2$

The BLUP technique was used to determine the EBVs in the MTDFREML package program. The breeding value of each animal was calculated using the formula below.

$BV_i = h^2 \text{ x } (P_i – P)$

BV_i: the breeding value of the *i*-th animal,

h^2: heritability of *i*-th animal,

P_i: the phenotypic value of the *i*-th animal in terms of the trait emphasized, P: population means in terms of the trait emphasized.

A one-way analysis of variance (ANOVA) was performed to examine the variation between genotypes in terms of EBVs. Tukey's multiple comparison test was used to compare the means of genotypes whose effect was found to be significant by analysis of variance.

Results

Association analysis, variance components, and breeding values

The effects of parity (**P**), calving year (**CY**), days in milk (**DIM**), A, C, and MT factors on LMY were significant, whereas the effects calving season (**CS**) and first calving age (**FCA**) were insignificant. Regarding A, the effects of P, CY, CS, DIM, MT, and LMY on A were significant. The C value, another milk yield trait, was considerably affected by the P, CY, DIM, and LMY, whereas the effects of CS and MT were determined to be insignificant (Supplementary Table S3). The effects of P, CY, DIM, A, and LMY factors on MT were statistically significant but C and CS had no effect. Another trait, LMY_{305}, was affected by factors P, CY, and FCA significantly, but the effect of CS was insignificant. In the current study, the P, CY, and FCA significantly affected LMY_{200}, whereas the effect of CS was found to be insignificant. In LMY_{100}, even though the effects of P, CY, and FCA on this milk yield trait were found to be significant, the effect of CS was not (Supplementary Table S4).

In the present study, the effects of P, CY, service period (**SP**), and LMY on DIM were found to be significant, whereas CS, and FCA did not affect this trait. The effects of P, CY, CS, and the milking time of peak milk yield (**MTPMY**) on PMY were found to be significant, whereas the effects of the activity of peak milk yield (**APMY**), the conductivity of peak milk yield (**CPMY**) and DPMY were not significant. A further trait DPMY, was significantly affected by the P, CY, and milking time of days to peak milk yield (**MTDPMY**), but not the effects of CS, PMY, the activity of days to peak milk yield (**ADPMY**) and conductivity of days to peak milk yield (**CDPMY**) (Supplementary Table S5). The P, CY, CS, A, C and MT had a significant effect on ADMY, but DIM and FCA had no effect. Another important milk yield feature DP was considerably influenced by the CY whereas not the effects of P, CS, LMY, DIM and calving interval (**CI**) (Supplementary Table S6).

Conclusions and suggestions

As a result, the associations between milk yield traits excluding activity and *Sau3A*I polymorphism were statistically insignificant in this study. Nevertheless, because the associations between activity and lameness or other health-related traits were not examined in this study, no allele or genotype was recommended for MAS-based selection studies for all-milk yield traits. According to our knowledge of the literature, the present study is the first to examine the associations between the *Sau3A*I polymorphism and MT, LMY_{200}, and DP. For the first time, the association between EBVs for milk yield traits and the *Sau3A*I polymorphism of the leptin gene was investigated in this study. Although there was no statistically significant difference in EBVs for the traits LMY, LMY_{305}, LMY_{200}, LMY_{100}, PMY, and ADMY, the genotypes with the highest values should be chosen in the selected studies. However, genotypes with the lowest breeding values should be chosen for C and DPMY. Besides, genotypes with the closest breeding values to 305 and 60 days, respectively, should be selected for DIM and DP. Because extremely high or low activity could indicate estrous, in the selection programs, genotypes with moderate breeding value in terms of A should be chosen to decrease lameness and other problems. Since extending the MT could cause udder tissue damage, it was found that the cattle with the AB genotype, which have a lower breeding value in terms of MT, can be used in selection to reduce the MT. However, the leptin gene should still be tested to decide whether to use it as a candidate gene in a herd.

Acknowledgments

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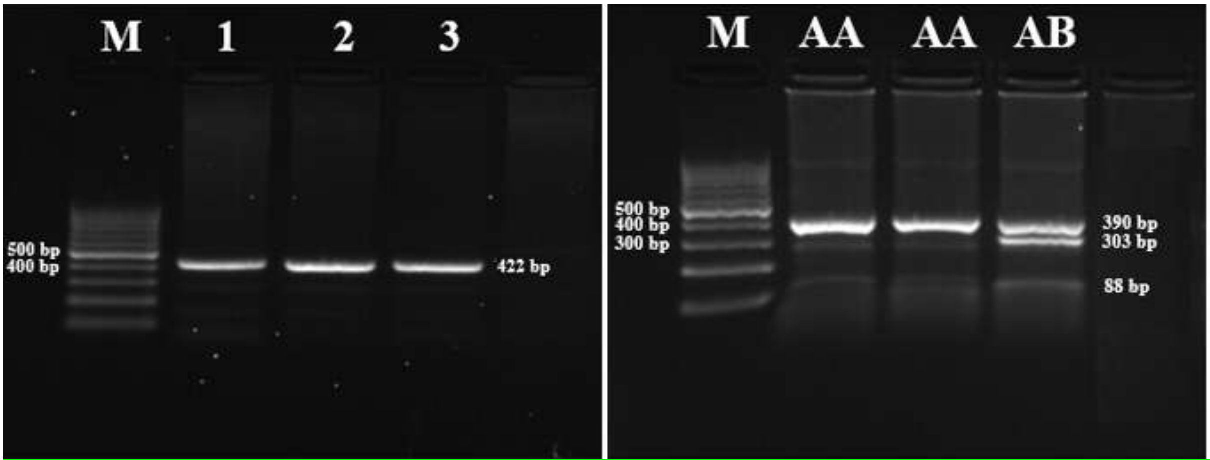
Supplementary Figure

Supplementary Figure S1. PCR and restriction products picture of the *Sau3A*I polymorphism



M: 100 bp Plus DNA ladder, Left image line 1-3: PCR products and Right image line AA: 390 and 32 bp, AB: 390, 303, 88 and 32 bp (32 bp was not seen in gel)

Supplementary Tables

Supplementary Table S1. Factors used to estimate variance components.

Milk yield traits	Discrete factors	Continuous factors
Lactation milk yield (kg)	Parity**, calving year**, calving season*	DIM**, C**, MT**, A*, FCA
Activity (step/day)	Parity*, calving year**, calving season**	DIM**, MT**, LMY**
Conductivity (m*S*/cm)	Parity**, calving year**, calving season	DIM**, MT, LMY*
Milking time (min)	Parity*, calving year**, calving season	DIM**, C, A**, LMY**
305 days of lactation milk yield (kg)	Parity**, calving year**, calving season	FCA*
200 days of lactation milk yield (kg)	Parity**, calving year**, calving season	FCA*
100 days of lactation milk yield (kg)	Parity**, calving year**, calving season	FCA**
Days in milk (day)	Parity**, calving year**, calving season	FCA, SP**, LMY**
Peak milk yield (kg)	Parity**, calving year**, calving season*	DPMY, APMY, CPMY, MTPMY*
Days to peak milk yield (day)	Parity**, calving year*, calving season	PMY, ADPMY, CDPMY, MTDPMY*
Average daily milk yield (kg)	Parity**, calving year**, calving season*	DIM, C*, MT**, A**, FCA
Dry period (day)	Parity, calving year**, calving season	LMY, DIM, CI

LMY: Lactation milk yield, A: Activity, C: Conductivity, MT: Milking time, DIM: Days in milk, PMY: Peak milk yield, DPMY: Days to peak milk yield, *: p<0.05, **: p<0.01, kg: Kilogram, m*S*/cm: Milli siemens/centimetre, FCA: First calving age, APMY: Activity of peak milk yield, CPMY: Conductivity of peak milk yield, MTPMY: Milking time of peak milk yield, ADPMY: Activity of days to peak milk yield, CDPMY: Conductivity of days to peak milk yield, MTDPMY: Milking time of days to peak milk yield

Supplementary Table S2. Allele and genotype frequencies and heterozygosity of Leptin gene polymorphism in Holstein-Friesian dairy cattle (Kibar & Aytekin 2021).

Leptin	**N**	**Genotypes**			**Genotype frequencies**			**Allele frequencies**		**χ^2**	**H_e**
		AA	**AB**	**BB**	**AA**	**AB**	**BB**	**A**	**B**		
Observed	212	162	50	0	0.764	0.236	0.000	0.8821	0.1179	3.789^{NS}	0.208
Expected	212	164.95	44.10	2.95	0.78	0.21	0.01				

N: Number of animals, χ^2 (HWE): Hardy-Weinberg equilibrium $\chi2$ value, H_e: Expected heterozygosity, NS: Not significant

Supplementary Table S3. The associations between lactation milk yield, activity and conductivity with *Sau3A*I polymorphism.

Factors		N	Traits		
			Lactation milk yield (kg)	Activity (step/day)	Conductivity (m*S*/cm)
			$\bar{X} \pm S_{\bar{X}}$	$\bar{X} \pm S_{\bar{X}}$	$\bar{X} \pm S_{\bar{X}}$
Leptin	AA	318	9687.84±111	170.86±2.35	10.15±0.04
	AB	112	9818.36±145	176.24±3.13	10.09±0.05
Parity	1	212	9525.6±115[C]	181.44±2.41[a]	9.64±0.04[C]
	2	95	10332.1±146[A]	172.85±3.15[ab]	10.06±0.05[B]
	3	59	10104.9±192[AB]	170.50±4.00[b]	10.30±0.07[A]
	4	33	9651.1±249[BC]	166.05±5.35[b]	10.24±0.09[AB]
	5	31	9151.8±262[C]	176.90±5.63[ab]	10.36±0.09[A]
Calving year	2013	26	8274.2±278[C]	170.13±6.37[AB]	9.99±0.10[AB]
	2014	38	9057.5±239[C]	176.60±5.24[A]	10.24±0.09[AB]
	2015	59	9976.1±202[B]	180.47±4.24[A]	10.28±0.07[A]
	2016	69	10131.3±175[B]	179.48±3.76[A]	10.09±0.06[AB]
	2017	101	10305.4±145[AB]	175.16±3.14[A]	10.00±0.05[B]
	2018	137	10774.0±142[A]	159.46±3.02[B]	10.12±0.05[AB]
Calving season	Winter	87	9685.8±168	171.90±3.65[AB]	10.10±0.06
	Spring	86	9515.3±165	179.45±3.52[A]	10.20±0.06
	Summer	141	9770.8±134	177.23±2.90[A]	10.03±0.05
	Autumn	116	10040.4±153	165.62±3.24[B]	10.15±0.05
Regression			DIM: 27.92±1.10** C: -383±139** MT: 378.8±59.3** A: -5.60±2.27* FCA: 1.44±1.06	DIM: 0.1076±0.0370** MT: 3.67±1.34** LMY: -0.00285±0.00105**	DIM: 0.002758±0.000603** MT: 0.0370±0.0218 LMY: -0.000044±0.000017*
R^2			0.6725	0.1492	0.3258

LMY: Lactation milk yield, A: Activity, C: Conductivity, DIM: Days in milk, MT: Milking time, FCA: First calving age, a, b: P<0.05, A, B: P<0.01, *: P<0.05, **: P<0.01, N: Number of animals, R^2: Coefficient of determination, $\bar{X}$: Least mean squares, $S_{\bar{X}}$: Standard error

Supplementary Table S4. The associations between Milking time, LMY_{305}, LMY_{200} and LMY_{100} with *Sau3A*I polymorphism.

Factors		N	Traits			
			Milking time (min)	LMY_{305}	LMY_{200}	LMY_{100}
			$\bar{X} \pm S_{\bar{X}}$	$\bar{X} \pm S_{\bar{X}}$	$\bar{X} \pm S_{\bar{X}}$	$\bar{X} \pm S_{\bar{X}}$
Leptin	AA	318	8.89±0.09	9159.71±114	6794.01±67.10	3528.29±32.00
	AB	112	8.71±0.12	8956.98±155	6777.30±91.50	3543.29±43.60
Parity	1	212	8.42±0.09^{B}	8559.25±113^{B}	6092.47±66^{C}	2988.01±31.60^{B}
	2	95	8.74±0.12^{AB}	9611.56±158^{A}	7122.76±93^{A}	3682.41±44.30^{A}
	3	59	8.80±0.15^{AB}	9596.91±200^{A}	7160.37±118^{A}	3753.84±56.20^{A}
	4	33	9.03±0.20^{A}	9027.01±266^{AB}	6903.53±157^{AB}	3666.12±74.70^{A}
	5	31	9.03±0.21^{A}	8497.00±274^{B}	6649.15±162^{B}	3588.60±77.00^{A}
Calving year	2013	26	8.99±0.23^{A}	7804.80±301^{D}	5733.93±177^{E}	3055.26±84.50^{D}
	2014	38	9.47±0.19^{A}	8522.30±250^{CD}	6474.09±147^{D}	3461.34±70.30^{C}
	2015	59	7.79±0.15^{C}	8894.60±203^{C}	6775.77±119^{CD}	3538.60±57.00^{BC}
	2016	69	8.32±0.14^{B}	9132.80±186^{C}	6970.89±110^{BC}	3621.34±52.30^{BC}
	2017	101	9.16±0.11^{A}	9740.10±152^{B}	7221.27±90^{B}	3683.56±42.80^{B}
	2018	137	9.08±0.11^{A}	10255.60±143^{A}	7537.97±84^{A}	3854.66±40.30^{A}
Calving season	Winter	87	8.88±0.13	9078.55±180	6744.08±106	3471.88±50.70
	Spring	86	8.80±0.13	8889.68±172	6739.36±101	3584.94±48.40
	Summer	141	8.76±0.11	8973.21±143	6721.39±85	3496.38±40.30
	Autumn	116	8.77±0.12	9291.96±162	6937.79±95	3589.98±45.50
Regression			DIM: -0.00756±0.00134** C: 0.189±0.110 A: 0.00492±0.00178** LMY: 0.000238±0.000037**	FCA: 2.50±1.13*	FCA: 1.521±0.663*	FCA: 0.707±0.316*
R^2			0.3167	0.2689	0.4105	0.5249

LMY_{305}: 305 days of lactation milk yield, LMY_{200}: 200 days of lactation milk yield, LMY_{100}: 100 days of lactation milk yield, DIM: Days in milk, C: Conductivity, A: Activity, LMY: Lactation milk yield, FCA: First calving age, A, B: P<0.01, *: P<0.05, **: P<0.01, N: Number of animals, R^2: Coefficient of determination, $\bar{X}$: Least mean squares, $S_{\bar{X}}$: Standard error

Supplementary Table S5. The associations between days in milk, peak milk yield and days to peak milk yield with *Sau3A*I polymorphism.

Factors		N	Traits		
			Days in milk (day)	Peak milk yield (kg)	Days to peak milk yield (kg)
			$\bar{X} \pm S_{\bar{X}}$	$\bar{X} \pm S_{\bar{X}}$	$\bar{X} \pm S_{\bar{X}}$
Leptin	AA	318	328.25±0.83	48.43±0.41	54.53±2.80
	AB	112	328.61±1.11	48.08±0.55	54.57±3.75
Parity	1	212	333.65±0.83^{A}	42.52±0.40^{B}	79.72±3.47^{A}
	2	95	329.83±1.11^{B}	49.48±0.56^{A}	51.26±3.84^{B}
	3	59	327.91±1.42^{BC}	50.67±0.71^{A}	51.85±4.90^{B}
	4	33	326.54±1.89^{BC}	49.53±0.94^{A}	44.85±6.42^{B}
	5	31	324.22±1.99^{C}	49.09±0.97^{A}	45.06±6.72^{B}
Calving year	2013	26	336.73±2.22^{A}	39.50±1.07^{E}	34.67±7.98^{b}
	2014	38	333.08±1.80^{AB}	46.50±0.89^{D}	53.17±6.31^{ab}
	2015	59	328.68±1.45^{BC}	47.87±0.71^{CD}	53.47±4.95^{ab}
	2016	69	324.64±1.32^{CD}	50.02±0.65^{BC}	58.89±4.51^{a}
	2017	101	324.64±1.08^{CD}	51.60±0.54^{B}	62.63±3.81^{a}
	2018	137	322.79±1.04^{D}	54.04±0.51^{A}	64.47±4.30^{a}
Calving season	Winter	87	329.41±1.29	47.85±0.63^{ab}	55.35±4.47
	Spring	86	328.75±1.25	48.13±0.61^{ab}	51.19±4.14
	Summer	141	327.49±1.03	47.68±0.51^{b}	59.03±3.62
	Autumn	116	328.06±1.14	49.37±0.58^{a}	52.62±3.96
Regression			FCA: -0.00123±0.00797 SP: 0.8323±0.0109** LMY: 0.003854±0.000328**	DPMY: -0.00715±0.00704 CPMY: 0.567±0.334 MTPMY: 0.2212±0.0896* APMY: -0.00722±0.00374	PMY: -0.245±0.347 CDPMY: -6.04±4.05 MTDPMY: -2.62±1.30* ADPMY: -0.0775±0.0444
R^2			0.9726	0.5732	0.2134

PMY: Peak milk yield, DPMY: Days to peak milk yield, FCA: First calving age, SP: Service period, LMY: Lactation milk yield, CPMY: Conductivity of peak milk yield, MTPMY: Milking time of peak milk yield, APMY: Activity of peak milk yield, CDPMY: Conductivity of days to peak milk yield, MTDPMY: Milking time of days to peak milk yield, ADPMY: Activity of days to peak milk yield, a, b: P<0.05, A, B: P<0.01, *: P<0.05, **: P<0.01, N: Number of animals, R^2: Coefficient of determination, $\bar{X}$: Least mean squares, $S_{\bar{X}}$: Standard error

Supplementary Table S6. The associations between ADMY and DP with *Sau3A*I polymorphism.

Factors		N	Traits		
			Average daily milk yield (kg)	N	Dry period (day)
			$\bar{X} \pm S_{\bar{x}}$		$\bar{X} \pm S_{\bar{x}}$
Leptin	AA	318	29.79±0.34	156	62.32±1.13
	AB	112	30.13±0.45	62	62.95±1.56
Parity	1	212	$29.19±0.35^{B}$	#	
	2	95	$31.67±0.45^{A}$	95	60.69±1.23
	3	59	$31.11±0.59^{A}$	59	61.15±1.50
	4	33	$29.68±0.77^{AB}$	33	62.07±2.01
	5	31	$28.15±0.81^{B}$	31	66.64±2.18
Calving year	2013	26	$25.40±0.86^{C}$	8	$52.89±3.71^{B}$
	2014	38	$27.58±0.74^{C}$	10	$61.66±3.27^{AB}$
	2015	59	$30.81±0.62^{B}$	38	$62.05±1.78^{AB}$
	2016	69	$31.18±0.54^{B}$	33	$66.60±1.83^{A}$
	2017	101	$31.71±0.45^{AB}$	58	$67.08±1.34^{A}$
	2018	137	$33.07±0.44^{A}$	71	$65.54±1.32^{A}$
Calving season	Winter	87	$29.68±0.52^{ab}$	46	62.96±1.74
	Spring	86	$29.26±0.51^{b}$	48	61.42±1.66
	Summer	141	$29.96±0.41^{ab}$	78	63.13±1.47
	Autumn	116	$30.94±0.47^{a}$	46	63.04±1.69
Regression			DIM: -0.00615±0.00337 C: -1.003±0.428* MT: 1.244±0.183** A: -0.01872±0.00700** FCA: 0.00541±0.00326	LMY: -0.000843±0.000447 DIM: 0.0191±0.0153 CI: 0.01439±0.00753	
R^2			0.3894	0.1335	

DIM: Days in milk, C: Conductivity, MT: Milking time, A: Activity, FCA: First calving age, LMY: Lactation milk yield, CI: Calving interval, a, b: P<0.05, A, B: P<0.01, *: P<0.05, **: P<0.01, N: Number of animals, R^2: Coefficient of determination, $\bar{X}$: Least mean squares, $S_{\bar{x}}$: Standard error

Supplementary Table S7. Variance components and heritability of milk yield traits.

Traits	Model								
	σ_a^2	$\sigma_a^2 x \sigma_m^2$	σ_m^2	σ_e^2	σ_p^2	h_a^2	h_m^2	r_{am}	-2 Log L
LMY (kg)	0.40665	0.15735	0.26318	0.87171	1.69889	0.24±0.173	0.16±0.167	0.48±0.136	642.2401958691
A (step/day)	0.02224	0.00615	0.00584	0.04599	0.08022	0.28±0.180	0.07±0.006	0.54±10.368	-599.3654385128
C (m*S*/cm)	0.09924	0.02055	0.00933	0.07012	0.19924	0.50±0.183	0.05±0.177	0.68±10.156	-330.2392815965
MT (min)	0.22618	0.18824	0.17591	0.46565	1.05598	0.21±0.143	0.17±0.146	0.94±0.237	420.7889335021
LMY_{305} (kg)	0.58191	0.02531	0.25066	1.10559	1.96347	0.30±0.164	0.13±0.010	0.07±0.791	689.9016060587
LMY_{200} (kg)	0.26977	0.01092	0.00995	0.37463	0.66527	0.41±0.177	0.01±0.001	0.21±20.079	234.6087479873
LMY_{100} (kg)	0.03157	0.01785	0.01009	0.09957	0.15909	0.20±0.132	0.06±0.005	1.00±10.478	-355.1561301709
DIM (day)	0.00616	0.00451	0.00330	0.00966	0.02363	0.26±0.273	0.14±0.026	1.00±10.996	-1300.134079639
PMY (kg)	0.06970	0.01269	0.00513	0.15040	0.23792	0.29±0.162	0.02±0.002	0.67±20.085	-168.2920895754
DPMY (day)	1.83023	0.42192	0.11560	9.43553	11.80328	0.16±0.117	0.01±0.104	0.92±40.610	1483.5616078396
ADMY (kg)	0.06453	0.06493	0.06532	0.06980	0.26458	0.24±0.153	0.25±0.031	1.00±0.990	-309.9723015443
DP (day)	0.03153	0.06321	0.12676	0.75765	0.97915	0.03±0.283	0.13±0.014	1.00±80.518	217.5937598359

LMY: Lactation milk yield, A: Activity, C: Conductivity, MT: Milking time, LMY_{305}: 305 days of lactation milk yield, LMY_{200}: 200 days of lactation milk yield, LMY_{100}: 100 days of lactation milk yield, DIM: Days in milk, PMY: Peak milk yield, DPMY: Days to peak milk yield, ADMY: Average daily milk yield, DP: Dry period, σ_a^2: Direct additive genetic variance, $\sigma_a^2 x \sigma_m^2$: Covariance between additive genetic effect and maternal effect, σ_m^2: Maternal genetic variance, σ_e^2: Temporary environmental variance σ_p^2: Phenotypic variance, h_a^2: Direct heritability, h_m^2: Maternal heritability, h_e^2: Heritability of environmental effect, r_{am}: Correlation between additive genetic effect and maternal effect, -2 Log L: Log-likelihood