Effects of the *DGAT1* polymorphism on test-day milk production traits throughout lactation

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ABSTRACT

Several studies have shown that the diacylglycerol O-acyltransferase 1 (*DGAT1*) K232A polymorphism has a major effect on milk production traits. It is less clear how effects of *DGAT1* on milk production traits change throughout lactation, if dominance effects of *DGAT1* are relevant, and whether *DGAT1* also affects lactose content, lactose yield, and total energy output in milk. Results from this study, using test-day records of 3 subsequent parities of around 1,800 cows, confirm previously reported effects of the *DGAT1* polymorphism on milk, fat, and protein yield, as well as fat and protein content. In addition, we found significant effects of the *DGAT1* polymorphism on lactose content and lactose yield. No significant effects on somatic cell score were detected. The effect of *DGAT1* on total energy excreted in milk was only significant in parity 1 and is mainly due to a higher energy output in milk of heterozygous cows. Significant but relatively small dominance effects of *DGAT1* on fat content and yield were detected, which are of little practical relevance. Significant *DGAT1* by lactation stage interaction was detected for milk yield, lactose yield, fat content, and protein content, indicating that the effect of the *DGAT1* polymorphism changes during lactation. In general, the *DGAT1* effect shows a large increase during early lactation (from the start of lactation to d 50 to 150) and tends to decrease later in lactation. No *DGAT1* by lactation stage interaction for fat yield was observed. Similar to *DGAT1*, effects of other genes also might vary throughout lactation and, therefore, using longitudinal models is recommended.

Key words: *DGAT1*, lactation stage, lactose, energy output

INTRODUCTION

Since the identification of the diacylglycerol O-acyltransferase 1 (*DGAT1*) K232A polymorphism by Grisart et al. (2002) and Winter et al. (2002), many studies have investigated associations between this polymorphism and milk production traits (e.g., Spelman et al., 2002; Thaller et al., 2003; Weller et al., 2003; Gautier et al., 2007). Although the magnitude of the estimated effects reported in these studies differ, they consistently show that the *DGAT1* K232A polymorphism has a major effect on milk production traits: the K allele is associated with a higher fat content, protein content, and fat yield, but lower milk and protein yields. Because of the strong negative relationship between lactose and fat content in milk across species (Fox, 2009), it is hypothesized that the *DGAT1* polymorphism also affects lactose content. To our knowledge, the effects of the *DGAT1* K232A polymorphism on lactose yield or content have not been quantified previously.

Most associations between the *DGAT1* K232A polymorphism and milk production traits are based on 305-d daughter yield deviations (Grisart et al., 2002; Winter et al., 2002; Spelman et al., 2002; Thaller et al., 2003; Weller et al., 2003; Gautier et al., 2007). Kuehn et al. (2007) detected significant dominance effects of the *DGAT1* polymorphism on milk fat content, and recent studies by Strucken et al. (2011) and Szyda et al. (2014) suggest that effects of *DGAT1* are not constant throughout lactation. Daughter yield deviations do not allow estimating dominance effects, and changes in gene effects throughout lactation remain unnoticed when effects are estimated based on 305-d production records. Consequently, dominant gene action of *DGAT1* has not been confirmed and the ambiguity on how effects of *DGAT1* on milk production traits change throughout lactation has not been resolved. Analysis based on test-day records throughout lactation would enable to study both phenomena.

Diacylglycerol O-acyltransferase 1 mediates the final step in triglyceride synthesis and is expressed in the

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small intestine, liver, adipose tissues, and the mammary gland (DeVita and Pinto, 2013; Muise et al., 2014). The pharmaceutical industry has a great interest in *DGAT1* as a target for human metabolic diseases. This interest was especially fueled by a study on *DGAT1* knockout mice that showed several beneficial metabolic phenotypes, including resistance to diet-induced obesity (DeVita and Pinto, 2013). Therefore, one might hypothesize an effect of *DGAT1* on traits other than milk production. Besides a direct effect of the *DGAT1* polymorphism on the cow metabolism, the strong effect of the *DGAT1* polymorphism on milk production traits might indirectly affect total energy output in milk and, consequently, the energy balance of cows. As a negative energy balance during early lactation of high-yielding dairy cows can result in metabolic and reproductive disorders, it is of interest to study the effect of the *DGAT1* polymorphism on total energy output in milk. The aim of the current study was to estimate the additive and dominance effects of the *DGAT1* polymorphism on milk production traits, including lactose and total energy output in milk, throughout the lactation in parity 1, 2, and 3 using test-day milk production records.

MATERIALS AND METHODS

Animals

Test day records from parity 1, 2, and 3 of cows involved in the Dutch Milk Genomics Initiative were retrieved from the database of the herd book (CRV, Arnhem, the Netherlands). The Dutch Milk Genomics Initiative comprised 2,000 first-lactation Dutch Holstein-Friesian cows from 398 herds throughout the Netherlands. At least 3 cows per herd were sampled. All cows were housed in loose housing systems, fed according to standard practice, and milked twice a day. Further details about the experimental design can be found in Stoop et al. (2008). Only animals that had been genotyped for the *DGAT1* K232A polymorphism were included in our study.

Traits

Fat, protein, and lactose content were based on infrared spectroscopy measurements using a MilkoScan FT6000 (Foss Electric, Hillerød, Denmark) at the Milk Control Station (Qlip, Zutphen, the Netherlands). Somatic cell count was determined using a Fossomatic 5000 (Foss Electric) at the Milk Control Station. Somatic cell counts were log-transformed (base 2) to obtain SCS. Fat, protein, and lactose yields per test day

were calculated by multiplying the respective contents with test-day milk yield. Total energy output in milk (TEM) per test day was calculated by multiplying the net energy for milk with test-day milk yield, where net energy for milk was calculated as described by Tyrrell and Reid (1965):

$$\begin{aligned} \text{TEM (MJ)} &= \text{net energy for milk (MJ/kg)} \\ &\times \text{kg of milk} = [0.384(\% \text{fat}) + 0.223 (\% \text{protein}) \\ &+ 0.199 (\% \text{lactose}) - 0.108] \times \text{kg of milk.} \quad [1] \end{aligned}$$

Genotypes

Genotypes for the *DGAT1* K232A polymorphism were obtained with a Taqman allelic discrimination assay in an Applied Biosystems 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA) as described by Schennink et al. (2007).

Statistical Analysis

Test-day records from parity 1, 2, and 3 were separately analyzed using the following repeatability model:

$$\begin{aligned} y_{ijklmno} &= \mu + \text{season}_i + \text{scode}_j + \text{lact}_k + \text{DGAT1}_l \\ &+ \beta_1 \text{ca}_{ijklmno} + \text{animal}_m + \text{HTM}_n + \text{ep}_o + e_{ijklmno}, \quad [2] \end{aligned}$$

where $y_{ijklmno}$ is a test-day observation; μ is the overall mean of the trait; season_i is the fixed effect of the i th class of calving season (4 classes: August–October, November–January, February–April, and May–July); scode_j is the fixed effect accounting for possible differences in genetic level between proven bull daughters and young bull daughters; lact_k is the fixed effect of lactation stage (26 classes of 15 d, with class 1 from 0 to 15 d and class 26 from 375 to 390 d in lactation); DGAT1_l is the fixed effect of *DGAT1* K232A genotype (AA, AK, or KK alleles); $\text{ca}_{ijklmno}$ is a covariate describing the effect of age at first, second, or third calving; animal_m is the random additive genetic effect of animal m ; HTM_n is the random effect of the n th herd-test-month; ep_o is the permanent environmental effect of cow o ; and $e_{ijklmno}$ is the random residual. Animal effects were assumed distributed as $N(\mathbf{0}, \mathbf{A}\sigma_a^2)$, herd-test-month effects as $N(\mathbf{0}, \mathbf{I}\sigma_{\text{HTM}}^2)$, permanent environmental effects as $N(\mathbf{0}, \mathbf{I}\sigma_{\text{ep}}^2)$, and residuals as $N(\mathbf{0}, \mathbf{I}\sigma_e^2)$, where $\mathbf{A}$ is the additive genetic relationships matrix and $\mathbf{I}$ the identity matrix. The $\mathbf{A}$ matrix was constructed based on 26,300 individuals.

Dominant mode of gene action was tested using the option !CONTRAST in ASReml v3.0 (Gilmour et al.,

Table 1. Descriptive statistics for test day records of 1,829 Dutch Holstein Friesian cows in parity 1, 1,578 cows in parity 2, and 1,204 cows in parity 3

Item	Parity 1			Parity 2			Parity 3		
	n	Mean	SD	n	Mean	SD	n	Mean	SD
Milk yield (kg)	19,593	24.6	5.3	15,793	29.0	8.2	11,329	30.8	9.5
Fat yield (kg)	19,547	1.06	0.22	15,752	1.24	0.33	11,284	1.31	0.38
Protein yield (kg)	19,547	0.85	0.17	15,752	1.02	0.24	11,284	1.07	0.28
Lactose yield (kg)	19,336	1.15	0.26	14,510	1.31	0.39	11,281	1.39	0.45
Fat (%)	19,547	4.36	0.65	15,752	4.37	0.72	11,284	4.34	0.73
Protein (%)	19,547	3.50	0.32	15,752	3.58	0.38	11,284	3.55	0.40
Lactose (%)	19,336	4.65	0.15	14,510	4.55	0.17	11,281	4.50	0.19
SCS ¹	18,240	6.00	1.49	14,908	6.33	1.68	10,813	6.79	1.76
TEM ² (MJ)	19,336	79.8	15.5	14,510	92.8	23.7	11,281	98.5	27.7

¹Log₂(SCC).²TEM (total energy output in milk) = kg of milk × [0.384 (% fat) + 0.223 (% protein) + 0.199 (% lactose) − 0.108].

2009). Dominance effects were defined as the deviation of the AK genotype effect from the average of KK and AA genotype effects. To investigate if the effect of the *DGAT1* polymorphism changed during lactation we tested for a *DGAT1* by lactation stage interaction using the following model:

$$\begin{aligned}
 y_{ijklmno} = & \mu + \text{season}_i + \text{scode}_j + \text{lact}_k + DGAT1_l \\
 & + (DGAT1 \times \text{lact})_{kl} + \beta_1 ca_{ijklmno} + \text{animal}_m \\
 & + \text{HTM}_n + \text{epo}_o + e_{ijklmno},
 \end{aligned} \quad [3]$$

where effects are as defined for model [2] and $(DGAT1 \times \text{lact})_{kl}$ is the *DGAT1* by lactation stage interaction.

RESULTS

Descriptive Statistics

Test-day records were available for 1,829 cows in parity 1. For a subset of 1,578 cows, parity 2 test-day records were also available, and for 1,204 of the cows, parity 3 test-day records were available for analysis. The frequency of the *DGAT1* K allele was 0.40 in parity 1 and 0.41 in parities 2 and 3. No significant deviation of the *DGAT1* genotype frequencies from Hardy-Weinberg equilibrium were noted in any of the parities. Table 1 shows the descriptive statistics of the test-day records. The number of test-day records per cow ranged from 8.9 for SCS in parity 3 to 10.7 for milk yield in parity 1. The average milk production per day increased from 24.6 kg in parity 1 to 29.0 kg in parity 2 and, subsequently, to 30.8 kg in parity 3. For fat, protein, and lactose yield, similar increases in means with increasing parity number were observed. Mean fat and protein percent increased from parity 1 to 2, but was slightly lower in parity 3 as compared with

parity 2. Lactose percent decreased from parity 1 to 2 and, subsequently, to parity 3, whereas SCS and TEM increased. Standard deviations for all traits increased with increasing parity number.

Lactation-Average *DGAT1* Effects

The *DGAT1* polymorphism showed highly significant effects on milk production traits (Table 2). The K allele was associated with lower milk, protein, and lactose yields, and higher fat yield and fat, protein, and lactose percent. No significant effect of the *DGAT1* polymorphism on SCS was detected. The effect of *DGAT1* on TEM was only significant in parity 1 ($P < 0.05$), where the AK genotype was associated with higher TEM. This effect would not be significant when adjusting for multiple testing.

Additive genetic effects for yield traits (kilograms of milk, fat, protein, and lactose) in parity 2 were about 30% higher than in parity 1 (Table 2). For fat and protein percent, this was about 18%. The additive effect of *DGAT1* on lactose percent was twice as large in parity 2 as in parity 1, and the effect further increased in parity 3. For most other traits, the effects of *DGAT1* in parity 3 tended to be slightly smaller than in parity 2. When expressed in phenotypic standard deviations (as given in Table 1), effects on fat and protein percent in parities 1, 2, and 3 were similar.

Dominance

Significant dominance effects of *DGAT1* on fat percent were detected in all 3 parities (Table 3). The estimated dominance effect for fat percent was about 0.05. For fat yield, a significant dominance effect was detected in parity 1 only. For TEM, a significant dominance effect was detected in parity 1, where TEM was

Table 2. Effect of the *DGAT1* K232A polymorphism on test-day milk production traits in parity 1, 2, and 3 of Dutch Holstein-Friesians

Item	Parity1			Parity2			Parity3		
	AA ¹	KK	–Log(P) ²	AA	KK	–Log(P)	AA	KK	–Log(P)
Milk yield (kg)	1.04 (0.19)	–1.45 (0.25)	17.9***	1.66 (0.27)	–1.66 (0.34)	17.3***	1.52 (0.35)	–1.53 (0.43)	9.2***
Fat yield (kg)	–0.068 (0.008)	0.018 (0.010)	20.7***	–0.074 (0.011)	0.042 (0.013)	15.5***	–0.080 (0.014)	0.046 (0.017)	10.6***
Protein yield (kg)	0.011 (0.007)	–0.032 (0.008)	5.0***	0.027 (0.009)	–0.031 (0.011)	5.1***	0.022 (0.011)	–0.024 (0.014)	1.9*
Lactose yield (kg)	0.045 (0.009)	–0.065 (0.012)	16.0***	0.070 (0.013)	–0.069 (0.016)	14.4***	0.061 (0.016)	–0.061 (0.020)	6.9***
Fat (%)	–0.45 (0.02)	0.36 (0.02)	169.8***	–0.51 (0.02)	0.44 (0.03)	172.1***	–0.50 (0.03)	0.39 (0.03)	113.7***
Protein (%)	–0.10 (0.01)	0.08 (0.01)	38.5***	–0.12 (0.01)	0.10 (0.02)	36.9***	–0.10 (0.01)	0.10 (0.02)	22.8***
Lactose (%)	–0.01 (0.01)	0.01 (0.01)	2.1**	–0.02 (0.01)	0.02 (0.01)	4.0***	–0.03 (0.01)	0.03 (0.01)	6.3***
SCS	0.04 (0.06)	0.00 (0.08)	0.1 ³	0.04 (0.07)	–0.09 (0.09)	0.3 ³	0.00 (0.09)	–0.01 (0.11)	0.0 ³
TEM ⁴ (MJ)	–1.61 (0.57)	–1.17 (0.73)	1.9*	–1.05 (0.79)	–0.42 (1.00)	0.4 ³	–1.54 (1.05)	0.17 (1.30)	0.5 ³

¹Estimates for AA and KK genotypes are expressed relative to the effect of the AK genotype, which is set to 0; SE in parentheses.²Significance levels are represented by $-\log_{10}(P\text{-value})$.³Not significant.⁴TEM (total energy output in milk) = kg of milk $\times$ [0.384 (% fat) + 0.223 (% protein) + 0.199 (% lactose) – 0.108].* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

highest for AK cows. No significant dominance effects of the *DGAT1* polymorphism were detected for the other traits.

DGAT1 by Lactation Stage Interaction

Significant *DGAT1* by lactation stage interactions were detected for milk yield, lactose yield, and fat and protein percent in all 3 parities (Table 3). This is illustrated in Figure 1, which shows the estimates of the (*DGAT1* $\times$ Lact) interaction term for parity 1 from a model without the main effects of *DGAT1*₁ and lact_k and the effect of the AK genotype in lactation stage 13 fixed at 0. These estimates have been used to calculate additive genetic effects at different stages of lactation, which were defined as half the difference between the KK and AA genotypic effects. Figure 2 shows the additive effects for parities 1 and 2. Results for parity 3 are not shown because these were very similar to results for parity 2.

The difference in milk yield between the 3 genotypes was small during the first 30 d of lactation (Figure 1 and 2). In parity 1, an additive effect of around –1.6 kg of milk was reached at d 100 in lactation and stayed approximately constant at this level until d 200 in lactation. Later in lactation (>200 d) the additive effect decreased until about –1 kg at d 300 (Figure 2). For parity 2 and 3, a similar pattern was observed, but differences between KK and AA genotypes were larger and the maximum difference was reached earlier in lactation (i.e., around 70 d in lactation). For lactation stages >300 d, larger fluctuations in the estimates were observed due to smaller numbers of observations.

Interestingly, no evidence was observed for a *DGAT1* by lactation stage interaction for fat yield (Table 3). Figure 2 shows that the additive effect of *DGAT1* on fat yield was rather constant during the lactation. In parity 1, a significant dominance effect was detected for fat yield, but also the dominance effect was constant throughout lactation (Figure 1). There was a highly significant *DGAT1* by lactation stage interaction on fat percent. At the start of the lactation (<30 d) the difference in fat percent between the KK and AA genotypes was approximately half of that in mid and late lactation (>150 d; Figure 2).

Significant *DGAT1* by lactation stage interactions for protein yield were found in parities 1 and 3. Similar to milk yield, there was a tendency of the *DGAT1* effect to increase during early lactation (<100 d) and to decrease later in lactation (Figure 2). For protein percent, a highly significant *DGAT1* by lactation stage interaction was noted, which showed a similar pattern as was observed for fat percent (Figure 2). The difference between the KK and AA genotypes was almost

Table 3. Dominance effects of the *DGAT1* K232A polymorphism and *DGAT1* by lactation stage interaction ($DGAT \times Lact$) on test-day milk production traits in parity 1, 2, and 3 of Dutch Holstein-Friesians

Item	Parity 1			Parity 2			Parity 3		
	Dominance		$DGAT \times Lact$	Dominance		$DGAT \times Lact$	Dominance		$DGAT \times Lact$
	d ¹	–Log(P) ²	–Log(P)	d	–Log(P)	–Log(P)	d	–Log(P)	–Log(P)
Milk yield (kg)	0.21 (0.18)	0.6 ³	27.4***	–0.00 (0.25)	0.0 ³	9.7***	0.01 (0.31)	0.0 ³	20.5***
Fat yield (kg)	0.025 (0.007)	3.5***	0.5 ³	0.016 (0.010)	1.0 ³	0.0 ³	0.017 (0.013)	0.8 ³	0.3 ³
Protein yield (kg)	0.011 (0.006)	1.1 ³	1.5*	0.002 (0.008)	0.1 ³	0.8 ³	0.001 (0.010)	0.0 ³	6.3***
Lactose yield (kg)	0.010 (0.008)	0.7 ³	23.1***	0.000 (0.011)	0.0 ³	8.9***	0.000 (0.014)	0.0 ³	16.4***
Fat (%)	0.05 (0.02)	2.1**	54.6***	0.04 (0.02)	1.3*	34.3***	0.05 (0.02)	1.7*	28.9***
Protein (%)	0.01 (0.01)	0.6 ³	74.4***	0.01 (0.01)	0.3 ³	18.1***	0.00 (0.01)	0.0 ³	6.3***
Lactose (%)	0.00 (0.00)	0.2 ³	0.5 ³	–0.00 (0.01)	0.2 ³	1.5*	–0.00 (0.01)	0.1 ³	3.3***
SCS	–0.02 (0.05)	0.1 ³	0.5 ³	0.03 (0.07)	0.2 ³	0.8 ³	0.00 (0.08)	0.0 ³	0.2 ³
TEM ⁴ (MJ)	1.39 (0.52)	2.1**	1.9*	0.73 (0.72)	0.5 ³	0.2 ³	0.69 (0.94)	0.3 ³	3.3***

¹d = dominance effect; SE in parentheses.
²Significance levels are represented by –log₁₀(P-value).
³Not significant.
⁴TEM (total energy output in milk) = kg of milk × [0.384 (% fat) + 0.223 (% protein) + 0.199 (% lactose) – 0.108].
*P < 0.05, **P < 0.01, ***P < 0.001.

absent in early lactation (<20 d) and increased until about 150 d in lactation with a tendency to decrease later in lactation.

The *DGAT1* by lactation stage interactions for lactose yield was similar to that for milk yield. In parity 1, no significant *DGAT1* by lactation stage interaction was seen for lactose content, but in parity 2 and 3 the effect of *DGAT1* on lactose content increased with lactation stage (Table 3 and Figure 2).

DISCUSSION

Results from our study confirmed previously reported effects of the *DGAT1* polymorphism on milk production traits. In addition, we found significant effects on lactose content and lactose yield but no significant effects on SCS. A significant effect on total energy excreted in milk was detected only in parity 1. In all 3 parities, significant *DGAT1* by lactation stage interactions were detected for milk yield, lactose yield, fat content, and protein content, indicating that the effect of the *DGAT1* polymorphism changes throughout lactation. Dominance effects were detected for fat content and for fat yield and TEM in parity 1 only.

Literature

Several studies have reported on the effects of the *DGAT1* polymorphism on milk production traits. Most of these studies estimated allele substitution effects using 305-d daughter yield deviations, which are often based on multiple parities. (e.g., Grisart et al., 2002; Winter et al., 2002; Spelman et al., 2002; Thaller et al., 2003; Weller et al., 2003; Gautier et al., 2007). This complicates comparing estimates obtained in different studies. The effects estimated in our study translate into a difference between AA and KK genotypes on a 305-d base of 774 kg of milk, –26.2 kg of fat, and 13.6 kg of protein in parity 1, 1,042 kg of milk, –35.8 kg of fat, and 18.0 kg of protein in parity 2, and 1,028 kg of milk, –37.2 kg of fat, and 16.4 kg of protein in parity 3. These results are in line with estimates by Grisart et al. (2002) and Gautier et al. (2007), but are considerably larger than estimates reported by some other studies (e.g., Spelman et al., 2002; Berry et al., 2010). Spelman et al. (2002) indicated that *DGAT1* effects in Dutch Holsteins and New Zealand Holsteins were similar when expressed in genetic standard deviations and, therefore, part of the differences in estimates might be attributed to scaling. In the current study, we found that effects of *DGAT1* were larger in parity 2 and 3 as compared with parity 1, which confirms results in German Holsteins (Thaller et al., 2003). When expressed in phenotypic standard deviations, effects in parities 1, 2, and 3 were

Figure 1. Estimates for *DGAT1* (AA, AK, and KK genotypes) by lactation stage interaction for parity 1 cows for milk, fat, and protein yields (in kilograms), fat, protein, and lactose percentages, SCS, and total energy output in milk (TEM, in MJ) and the significance of the effect of *DGAT1* and *DGAT1* × Lact). Color version available online.

Figure 2. The additive effect $[(KK - AA)/2]$ of the *DGAT1* polymorphism on test-day milk production traits for different lactation stages in parity 1 and 2. Color version available online.

similar. This illustrates the effect of scaling on *DGAT1* effects.

Lactose and SCS

In our study, we also showed significant effects of *DGAT1* on lactose yield and content, where the K allele was associated with a lower lactose yield and higher

lactose content. To our knowledge, estimates of *DGAT1* on lactose content and yield have not been reported previously. Association of the *DGAT1* K allele with higher contents of both fat and lactose is remarkable, as across species there is a strong negative relationship between lactose and fat content in milk (Fox, 2009). Lactose is the major solute in milk and osmolarity of milk is bound by biological constraints; therefore, lactose con-

tent of milk shows very little variation (e.g., Stoop et al., 2007). To keep the osmolarity of milk constant, the effect of *DGAT1* on lactose content is probably accompanied by effects on Na^+ , K^+ , or Cl^- (i.e., other major contributors to the osmolarity of milk).

We observed that the effect of *DGAT1* on lactose content increased with parity and this increase cannot be explained by scaling. This increased effect in later parities could be related to higher incidence of mastitis, as mastitis is known to be associated with lower lactose contents. However, we did not observe significant effects of *DGAT1* on SCS, which is an indicator for udder health and mastitis. This is in contrast with Barbosa da Silva et al. (2010), who reported a significant effect of the *DGAT1* polymorphism on SCS, and with Mach et al. (2012), who reported that the *DGAT1* K232A polymorphism affected the expression of several genes involved in the immune system and associated with bovine mastitis.

Total Energy Output

Despite the dramatic effects of *DGAT1* genotypes on milk yield and composition, the effect on total energy output in milk is small or absent. The only evidence we found was an increased energy output of AK cows in parity 1. This indicates that energy requirements for milk production hardly differ between cows with different *DGAT1* genotypes. Therefore, we also do not expect large differences in (negative) energy balance between these cows. This is in agreement with Banos et al. (2008), who concluded that *DGAT1* K has only a marginal positive effect on cumulative effective energy balance.

Several studies have reported associations between the *DGAT1* polymorphism and reproductive traits; however, results are not conclusive. Kaupe et al. (2007) reported a negative effect of the *DGAT1* K allele on nonreturn rates, whereas Oikonomou et al. (2009) reported that the K allele was associated with less inseminations and higher conception rates. Based largely on the same first-parity data as used in the current study, Demeter et al. (2009) suggested a nonadditive effect of *DGAT1*, with AK cows having lower (6 and 4%) nonreturn rates at 28 and 56 d after first service. In the current study, *DGAT1* AK cows had significantly higher values for TEM than AA or KK cows. This suggests that AK cows in first parity might have a higher negative energy balance than AA or KK cows. This might explain the suggestive effects of *DGAT1* on nonreturn rates reported by Demeter et al. (2009). In parities 2 and 3, we did not detect an effect of *DGAT1* on TEM; therefore, the effect of *DGAT1* on fertility might be limited to first-parity cows.

Dominance

We detected significant dominance effects for *DGAT1* on fat content in all 3 parities and for fat yield in parity 1. Kuehn et al. (2007) reported a dominance effect of 0.057 for fat content, which is close to our estimate. In agreement with dominance gene action on fat yield, Strucken et al. (2011) reported significant differences in fat yield between AA and AK genotypes but a nonsignificant difference between genotype AK and KK. The dominance effects on fat content and yield are relatively small and of little practical relevance from a breeding perspective. However, from a biological perspective it is an interesting observation. Grisart et al. (2004) showed that the amount of triglycerides synthesized by the K allele is about 1.5 times the amount synthesized by the A allele, suggesting that the V_{max} [enzymatic reaction, at maximum (saturating) substrate concentrations] of the *DGAT1* K allele is higher than that of the A allele. A nonlinear relationship between the amount of enzyme and the amount of product is the basis of the classical explanation of dominance (Wright, 1934); therefore, this might provide an explanation for the observed dominance effects on fat content and fat yield.

Interaction with Lactation Stage

The results of our study show that the effect of the *DGAT1* polymorphism on milk yield, lactose yield, fat content, and protein content is not constant throughout lactation. These results support the basic findings by Strucken et al. (2011) and Szyda et al. (2014); however, some differences exist on how effects of *DGAT1* on milk production traits change throughout lactation. Strucken et al. (2011) concluded that the characteristic *DGAT1* genotypic effects occur after lactation d 40. In the current study, we observed that the lactation stage at which the maximum difference between *DGAT1* genotypes is reached differed between parities and traits and ranges between d 50 and 150 in lactation. Szyda et al. (2014) concluded that effects of *DGAT1* on fat and protein content increased during lactation, whereas we observed an increase in effects of *DGAT1* on fat and protein content during the first 150 d in lactation, but later in lactation the effects stabilized for fat content and decreased for protein content. Furthermore, Szyda et al. (2014) reported that effects of *DGAT1* on milk yield were constant throughout lactation, whereas we observed a strong *DGAT1* by lactation stage interaction for milk yield.

The *DGAT1* by lactation stage interaction is the main reason for differences between estimated *DGAT1* effects reported in the current study and our earlier

studies. Based on largely the same first-parity animals, we previously reported a difference between *DGAT1* KK and AA genotypes for fat content of about 1% (Schennink et al., 2008; Duchemin et al., 2013). In the current study, the estimated difference between KK and AA genotypes in first-parity Dutch Holstein Friesians is approximately 0.8% (Table 2), which is considerably lower than the previously reported estimate. Milk samples included in the studies of Schennink et al. (2008) and Duchemin et al. (2013) were based on a single test-day record of cows between 63 and 282 d in lactation, whereas our study includes samples of cows in early lactation when effects of *DGAT1* on fat content are smaller.

Several quantitative genetic studies showed that additive genetic variance changes throughout lactation and genetic correlations between test-day milk production records differ from unity (e.g., Druet et al., 2003; Caccamo et al., 2008). This indicates that effects of genes change during lactation, which is confirmed by gene expression studies (e.g., Bionaz and Loor, 2008; Bionaz et al., 2012; Wickramasinghe et al., 2012; Gao et al., 2013). Bionaz et al. (2012) provided evidence for differential expression of *DGAT1* during lactation and indicated that significant changes in *DGAT1* expression only occurred in early lactation (<15 d), whereas later in lactation no significant changes in *DGAT1* expression were detected. In addition to differences in expression of *DGAT1* throughout lactation, another DGAT enzyme, *DGAT2*, also catalyzes the formation of triglycerides (Cases et al., 2001). Several studies have shown that *DGAT1* and *DGAT2* are both expressed in the bovine mammary gland and expression of both genes changes with the initiation of lactation (Bionaz and Loor, 2008; Bionaz et al., 2012; Wickramasinghe et al., 2012; Gao et al., 2013). Therefore, it seems likely that both are involved in milk FA synthesis, although little is known about their relative contribution to milk fat synthesis during lactation. Wickramasinghe et al. (2012) indicate that *DGAT1* had higher expression at d 90 in lactation than at d 15 or 250, and expression of *DGAT2* gradually increased from d 15 to 250. Bionaz and Loor (2008) also show differences in expression patterns during lactation for *DGAT1* and *DGAT2*. These studies indicate that expression of *DGAT1* is not constant throughout lactation and that the relative contributions of *DGAT1* and *DGAT2* to milk FA synthesis change during lactation. However, these expression studies are based on a limited number of sample points during lactation. Therefore, these studies do not allow us to conclude whether changes in expression of *DGAT1* or differences in relative contributions of *DGAT1* and *DGAT2* agree with the differences in estimated *DGAT1* effects on

milk production traits throughout lactation and, thus, might be a cause for the *DGAT1* by lactation stage interaction.

In addition to gene expression differences throughout lactation, the supply of FA to the udder also differs between early, mid-, and late lactation. The substrate available for esterification to the sn-3 position of a diacylglycerol, therefore, differs and this might have different effects on the *DGAT1* A and K variants. At the initiation of lactation cows are, in general, in negative energy balance and body fat is mobilized (Garnsworthy et al., 2006).

Most of the effects of *DGAT1* on milk production traits originate from the effect on water excretion (or dilution effect) and de novo FA synthesis (e.g., Schennink et al., 2008). Interestingly, we found that the additive effect of *DGAT1* on fat yield is rather constant throughout lactation (Figure 2) and it seems that especially the dilution effect is responsible for the *DGAT1* by lactation stage interaction. We concluded that the observed *DGAT1* by lactation stage interaction might be due to a change in expression of *DGAT1*, an interaction of *DGAT1* with other genes, or the supply of FA. The exact mechanism behind the observed effects, however, remains unclear.

Implications

We showed that the *DGAT1* K232A polymorphism has major effects on milk yield and composition. However, the effect of *DGAT1* on total energy excreted in milk is small. These results, therefore, do not give reason to assume that energy balance and associated metabolic and reproductive disorders are strongly affected by *DGAT1*. We also did not find a significant effect of *DGAT1* on SCS and, therefore, do not expect any association between *DGAT1* and the susceptibility to mastitis. Significant but relatively small dominance effects of *DGAT1* on fat content and yield were detected, which are of little practical relevance. Furthermore, significant *DGAT1* by lactation stage interactions were detected for several milk production traits. The exact mechanisms behind these changes in *DGAT1* effects sizes throughout lactation remain largely unknown; however, the magnitude of the changes in effect sizes are considerable. Lund et al. (2008) indicated that analyzing 305-d milk yield in QTL analyses maybe reasonable if the gene effect is constant throughout lactation. However, when the QTL effect changes during lactation longitudinal models or analysis using only data collected during part of the lactation might substantially increase QTL detection power. For *DGAT1*, a higher detection power can be obtained when milk samples in

mid- and late lactation are used. Effects of other genes also might vary throughout lactation and, therefore, estimating effects separately for parts of the lactation (e.g., early, mid-, and late lactation) or using longitudinal models is recommended.

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