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ABSTRACT

Several studies have shown that the DGAT1 K232A polymorphism has a major impact 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 three subsequent parities of around 1800 cows, confirm previously reported effects of the DGAT1 polymorphism on milk-, fat- and protein yield, and fat and protein content. In addition, we found significant effects of the DGAT1 polymorphism on lactose content and lactose yield. No significant effects on SCS 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 (AK) 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 (till d50 to d150) and tends to decrease later in lactation. There was no DGAT1 by lactation stage interaction for fat yield. Similar to DGAT1, effects of other genes also might vary throughout lactation and, therefore, using longitudinal models is recommended.

Keywords: 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; Weller et al. 2002; Thaller 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 impact 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-day daughter yield deviations (Grisart et al. 2002; Winter et al. 2002; Spelman et al. 2002; Weller et al. 2002; Thaller 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-day 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.

77 DGAT1 mediates the final step in triglyceride synthesis and is expressed in the small intestine,
78 liver, adipose tissues and the mammary gland (DeVita and Pinto, 2013; Muise et al. 2014). The
79 pharmaceutical industry has a great interest in DGAT1 as a target for human metabolic diseases.
80 This interest was especially fuelled by a study on DGAT1 knock-out mice which showed several
81 beneficial metabolic phenotypes, among others, resistance to diet induced obesity (DeVita and
82 Pinto, 2013). Therefore, one might hypothesize an effect of DGAT1 on traits other than milk
83 production. Besides a direct effect of the DGAT1 polymorphism on the cow's metabolism, there
84 also might be an indirect effect: the strong effect of the DGAT1 polymorphism on milk production
85 traits might affect total energy output in milk and, consequently, the energy balance of cows. Since
86 a negative energy balance during early lactation of high-yielding dairy cows can result in metabolic
87 and reproductive disorders, it is of interest to study the effect of the DGAT1 polymorphism on total
88 energy output in milk.

89 The aim of the current study was to estimate the additive and dominance effects of the DGAT1
90 polymorphism on milk production traits including lactose and total energy output in milk
91 throughout the lactation in parity 1, 2 and 3 using test-day milk production records.

92

93 **MATERIALS AND METHODS**

94 **Animals.** Test day records from parity 1, 2 and 3 of cows involved in the Dutch Milk Genomics
95 Initiative were retrieved from the data base of the herd book (CRV, Arnhem, the Netherlands). The
96 Dutch Milk Genomics Initiative comprised 2,000 first-lactation Dutch Holstein-Friesian cows from
97 398 herds throughout the Netherlands. At least three cows per herd were sampled. All cows were
98 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 which had been genotyped for the DGAT1 K232A polymorphism were included in this 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 Somatic Cell Scores (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 milk (NEm) with test-day milk yield, where NEm was calculated as described by Tyrrell and Reid (1965):

$$\text{TEM (MJ)} = \text{NEm (MJ/kg)} * \text{kg milk} =$$

$$[0.384(\%\text{fat}) + 0.223\ (\%\text{protein}) + 0.199\ (\%\text{lactose}) - 0.108] * \text{kg milk} \qquad [1]$$

Genotypes. Genotypes for the DGAT1 K232A polymorphism were obtained with a Taqman allelic discrimination assay 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:

$$y_{ijklmno} = \mu + season_i + scode_j + lact_k + DGAT1_l + \beta_1 ca_{ijklmno} + animal_m + HTM_n + ep_o + e_{ijklmno} \qquad [2]$$

where $y_{ijklmno}$ is a test-day observation. The overall mean of the trait is μ; $season_i$ is the fixed effect of the i^{th} class of calving season (four 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 days, with class 1 from 0-15 days till class 26 from 375-390 days in lactation), $DGAT1_l$ is the fixed effect of DGAT1 K232A genotype (AA, AK or KK); $ca_{ijklmno}$ is a covariate describing the effect of age at 1st, 2nd or 3rd 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 to be distributed as $N(\mathbf{0}, \boldsymbol{A}\sigma_a^2)$, Herd-Test-Month effects as $N(\mathbf{0}, \boldsymbol{I}\sigma_{HTM}^2)$, permanent environmental effects as $N(\mathbf{0}, \boldsymbol{I}\sigma_{Ep}^2)$, and residuals as $N(\mathbf{0}, \boldsymbol{I}\sigma_e^2)$, where **A** is the additive genetic relationships matrix and **I** the identity matrix. The **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. 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:

$$y_{ijklmno} = \mu + season_i + scode_j + lact_k + DGAT1_l + (DGAT1 \times lact)_{kl} + \beta_1 ca_{ijklmno} + animal_m + HTM_n + ep_o + e_{ijklmno} \quad [3]$$

where effects are as defined for model [2] and $(DGAT1 \times lact)_{kl}$ 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 also parity 2 test day records were 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. There was no significant deviation of the DGAT1 genotype frequencies from Hardy-Weinberg equilibrium 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% increased from parity 1 to parity 2 but was slightly lower in parity 3 as compared to parity 2. Lactose% decreased from parity 1 to parity 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, fat%, protein% and lactose%. 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 (kg milk, fat, protein and lactose) in parity 2 were about 30% higher than in parity 1 (Table 2). For fat% and protein% this was about 18%. The additive effect of DGAT1 on lactose% was twice as big 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 (SD as given in Table 1), effects on fat% and protein% in parities 1, 2 and 3 were similar.

Dominance. Significant dominance effects of DGAT1 on fat% were detected in all three parities (Table 3). The estimated dominance effect for fat% 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 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, fat% and protein% in all three parities (Table 3). This is illustrated in Figure 1, which shows the estimates of the (DGAT1 x lact) interaction term for parity 1 from a model without the main effects of $DGAT1_l$ 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 parity 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 days of lactation (Figure 1 and 2). In parity 1 an additive effect of around -1.6 kg of milk was reached at day 100 in lactation and stayed approximately constant at this level till day 200 in lactation. Later in lactation (>200d) the additive effect decreased till about -1 kg at day 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 days in lactation. For lactation stages >300 days larger fluctuations in the estimates were observed due to smaller numbers of observations.

185 Interestingly there was no evidence for a DGAT1 by lactation stage interaction for fat yield (Table
186 3). Figure 2 shows that the additive effect of DGAT1 on fat yield was rather constant during the
187 lactation. In parity 1 a significant dominance effect was detected for fat yield but also the
188 dominance effect was constant throughout lactation (Figure 1). There was a highly significant
189 DGAT1 by lactation stage interaction on fat%. At the start of the lactation (<30d) the difference in
190 fat% between the KK and AA genotypes was approximately half of that in mid and late lactation
191 (>150d; Figure 2).

192 Significant DGAT1 by lactation stage interactions for protein yield were found in parities 1 and 3.
193 Similar as for milk yield, there was a tendency of the DGAT1 effect to increase during early
194 lactation (<100 days) and to decrease later in lactation (Figure 2). For protein% there was a highly
195 significant DGAT1 by lactation stage interaction which showed a similar pattern as was observed
196 for fat% (Figure 2). The difference between the KK and AA genotypes was almost absent in early
197 lactation (<day 20) and increased till about 150d in lactation with a tendency to decrease later in
198 lactation.

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

203

204 **DISCUSSION**

205 Results from this study confirm previously reported effects of the DGAT1 polymorphism on milk
206 production traits. In addition, we found significant effects on lactose content and lactose yield but
207 no significant effects on SCS. A significant effect on total energy excreted in milk was detected

only in parity 1. In all three 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-day daughter yield deviations which are often based on multiple parities. (e.g. Grisart et al. 2002; Winter et al. 2002; Spelman et al. 2002; Weller et al. 2002; Thaller et al. 2003; Gautier et al., 2007). This complicates comparing estimates obtained in different studies. The effects estimated in this study translate into a difference between AA and KK genotypes on a 305-day base of +774 kg milk,-26.2 kg fat and +13.6 kg protein in parity 1, +1042 kg milk,-35.8 kg fat and +18.0 kg protein in parity 2, and +1028 kg milk,-37.2 kg fat and +16.4 kg 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 to 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 similar. This illustrates the impact of scaling on DGAT1 effects.

Lactose and SCS. In this 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 and, therefore, lactose content 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, since 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.

A number of 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 non-return rates whereas Oikonomou et al. (2009) reported that the K allele was associated with less inseminations and higher conception rates. Based on largely the same first parity data as used in the current study Demeter et al. (2009) suggested a non-additive effect of DGAT1 with AK cows having lower (6 and 4%) non return rates at 28 and 56 days 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 NEB than AA or KK cows. This might explain the suggestive effects of DGAT1 on non-return rates reported by Demeter et al. (2009). In party 2 and 3 we did not detect an effect of DGAT1 on TEM and, 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 three parities and for fat yield in parity 1. Kuenh 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 non-significant 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 Vmax of the DGAT1 K allele is higher than that of the A allele. A non-linear relationship between the amount of enzyme and the amount of product is the basis of the classical explanation of dominance (Wright, 1934) and, therefore, this might provide an explanation for the observed dominance effects on fat content and fat yield.

Interaction with lactation stage. The results of this 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, there are some differences 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 day 40. In the current study we observe that the lactation stage at which the maximum difference between DGAT1 genotypes is reached differs between parities and traits and ranges between day 50 and 150 in lactation. Szyda et al. (2014) concluded that effects of DGAT1 on fat and protein content increased during lactation while we observed an increase in effects of DGAT1 on fat and protein content during the first 150 days in lactation but later in lactation the effects stabilized for fat content and decreased for protein content. Further, Szyda et al. (2014) reported that effects of DGAT1 on milk yield were constant throughout lactation while we observe 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 this 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 days in lactation, whereas this study includes also 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

303 differential expression of DGAT1 during lactation and indicated that significant changes in DGAT1
304 expression only occurred in early lactation (<d15) whereas later in lactation no significant changes
305 in DGAT1 expression were detected. In addition to differences in expression of DGAT1 throughout
306 lactation, there is another DGAT enzyme, DGAT2, that also catalyzes the formation of triglycerides
307 (Cases et al. 2001). Several studies have shown that DGAT1 and DGAT2 are both expressed in the
308 bovine mammary gland and expression of both genes changes with the initiation of lactation
309 (Bionaz and Loor, 2008; Bionaz et al. 2012; Wickramasinghe et al. 2012; Gao et al. 2013).
310 Therefore, it seems likely that both are involved in milk fatty acid synthesis although little is known
311 about their relative contribution to milk fat synthesis during lactation. Wickramasinghe et al. (2012)
312 indicate that DGAT1 had higher expression at day 90 in lactation than at day 15 or 250, and
313 expression of DGAT2 gradually increased from day 15 till day 250. Bionaz and Loor (2008) also
314 show differences in expression patterns during lactation for DGAT1 and DGAT2. These studies
315 indicate that expression of DGAT1 is not constant throughout lactation and that the relative
316 contributions of DGAT1 and DGAT2 to milk FA synthesis change during lactation. However, these
317 expression studies are based on a limited number of sample points during lactation. Therefore, these
318 studies do not enable to conclude whether changes in expression of DGAT1 or differences in
319 relative contributions of DGAT1 and DGAT2 agree with the differences in estimated DGAT1
320 effects on milk production traits throughout lactation and, thus, might be a cause for the DGAT1 by
321 lactation stage interaction.

322 In addition to gene expression differences throughout lactation, the supply of FA to the udder also
323 differs between early, mid and late lactation. The substrate available for esterification to the sn-3
324 position of a diacylglycerol, therefore, differs and this might have different effects on the DGAT1 A
325 and K variants. At the initiation of lactation cows are in general in negative energy balance and
326 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 fatty acid 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 conclude 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. The effect of DGAT1 on total energy excreted in milk is, however, small. These results, therefore, do not give reason to assume that the 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. Further, 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-day 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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464 **Table 1.** Descriptive statistics for test day records of 1,829 Dutch Holstein Friesian cows in parity
465 1, 1,578 cows in parity 2 and 1,204 cows in parity 3

	Parity 1			Parity 2			Parity 3		
	n	Mean	(SD)	n	Mean	(SD)	n	Mean	(SD)
Milk Yield (kg)	19593	24.6	(5.3)	15793	29.0	(8.2)	11329	30.8	(9.5)
Fat Yield (kg)	19547	1.06	(0.22)	15752	1.24	(0.33)	11284	1.31	(0.38)
Protein Yield (kg)	19547	0.85	(0.17)	15752	1.02	(0.24)	11284	1.07	(0.28)
Lactose Yield (kg)	19336	1.15	(0.26)	14510	1.31	(0.39)	11281	1.39	(0.45)
Fat%	19547	4.36	(0.65)	15752	4.37	(0.72)	11284	4.34	(0.73)
Protein%	19547	3.50	(0.32)	15752	3.58	(0.38)	11284	3.55	(0.40)
Lactose%	19336	4.65	(0.15)	14510	4.55	(0.17)	11281	4.50	(0.19)
SCS[1]	18240	6.00	(1.49)	14908	6.33	(1.68)	10813	6.79	(1.76)
TEM (MJ)[2]	19336	79.8	(15.5)	14510	92.8	(23.7)	11281	98.5	(27.7)

466 [1]$\log_2$(Somatic Cell Count)

467 [2]kg milk * [0.384(%fat) + 0.223 (%protein) + 0.199 (%lactose) − 0.108]

468

469

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

	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	ns	$0.04_{(0.07)}$	$-0.09_{(0.09)}$	0.3	ns	$0.00_{(0.09)}$	$-0.01_{(0.11)}$	0.0	ns
TEM (MJ)	$-1.61_{(0.57)}$	$-1.17_{(0.73)}$	1.9	*	$-1.05_{(0.79)}$	$-0.42_{(1.00)}$	0.4	ns	$-1.54_{(1.05)}$	$0.17_{(1.30)}$	0.5	ns

[1]Estimates for AA and KK genotypes are expressed relative to the effect of the AK genotype which is set to 0, SE in parentheses

[2] Significance levels are represented by $-\log_{10}$(P-value), * $P<0.05$, ** $P<0.01$, *** $P<0.001$, ns: not significant

Table 3. Dominance effects of the DGAT1 K232A polymorphism and DGAT1 by lactation stage interaction on test-day milk production traits in parity 1, parity 2 and parity 3 of Dutch Holstein Friesians

	Parity 1			Parity 2			Parity 3		
	Dominance		DGATxLa	Dominance		DGATxLa	Dominance		DGATxLa
	d[1]	-Log(p)[2]	-Log(p)	d	-Log(p)[2]	-Log(p)	d	-Log(p)	-Log(p)
Milk Yield (kg)	$0.21_{(0.18)}$	0.6 ns	27.4 ***	$-0.00_{(0.25)}$	0.0 ns	9.7 ***	$0.01_{(0.31)}$	0.0 ns	20.5 ***
Fat Yield (kg)	$0.025_{(0.007)}$	3.5 ***	0.5 ns	$0.016_{(0.010)}$	1.0 ns	0.0 ns	$0.017_{(0.013)}$	0.8 ns	0.3 ns
Protein Yield (kg)	$0.011_{(0.006)}$	1.1 ns	1.5 *	$0.002_{(0.008)}$	0.1 ns	0.8 ns	$0.001_{(0.010)}$	0.0 ns	6.3 ***
Lactose Yield (kg)	$0.010_{(0.008)}$	0.7 ns	23.1 ***	$0.000_{(0.011)}$	0.0 ns	8.9 ***	$0.000_{(0.014)}$	0.0 ns	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 ns	74.4 ***	$0.01_{(0.01)}$	0.3 ns	18.1 ***	$0.00_{(0.01)}$	0.0 ns	6.3 ***
Lactose%	$0.00_{(0.00)}$	0.2 ns	0.5 ns	$-0.00_{(0.01)}$	0.2 ns	1.5 *	$-0.00_{(0.01)}$	0.1 ns	3.3 ***
SCS	$-0.02_{(0.05)}$	0.1 ns	0.5 ns	$0.03_{(0.07)}$	0.2 ns	0.8 ns	$0.00_{(0.08)}$	0.0 ns	0.2 ns
TEM (MJ)	$1.39_{(0.52)}$	2.1 **	1.9 *	$0.73_{(0.72)}$	0.5 ns	0.2 ns	$0.69_{(0.94)}$	0.3 ns	3.3 ***

[1] d: dominance effect, SE in parentheses

[2] Significance levels are represented by $-\log_{10}$(P-value), * $P<0.05$, ** $P<0.01$, *** $P<0.001$, ns: not significant

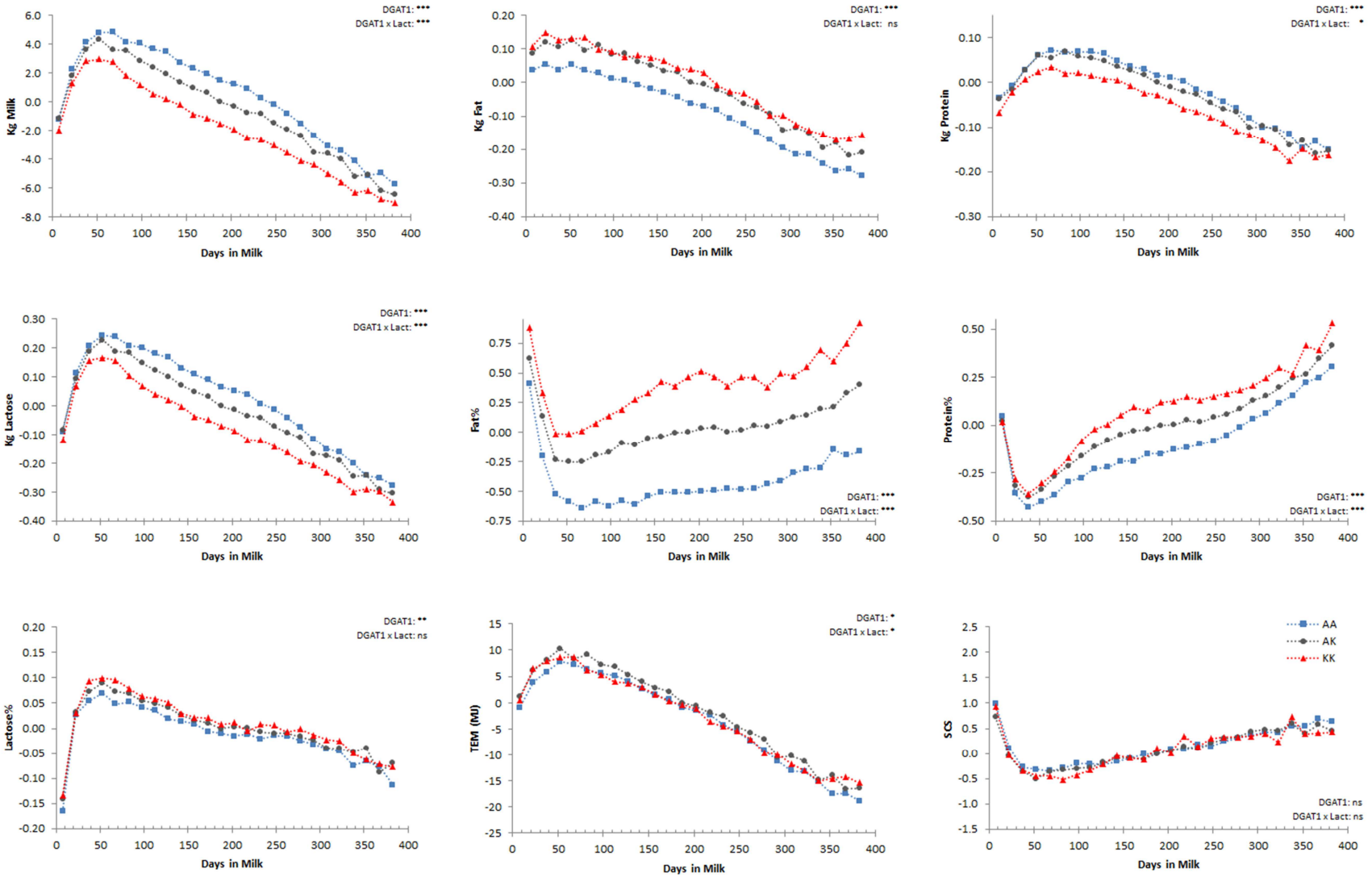
Kg Milk
DGAT1: ***
DGAT1 x Lact: ***
Days in Milk
Kg Fat
DGAT1: ***
DGAT1 x Lact: ns
Days in Milk
Kg Protein
DGAT1: ***
DGAT1 x Lact: *
Days in Milk
Kg Lactose
DGAT1: ***
DGAT1 x Lact: ***
Days in Milk
Fat%
DGAT1: ***
DGAT1 x Lact: ***
Days in Milk
Protein%
DGAT1: ***
DGAT1 x Lact: ***
Days in Milk
Lactose%
DGAT1: **
DGAT1 x Lact: ns
Days in Milk
TEM (MJ)
DGAT1: *
DGAT1 x Lact: *
Days in Milk
SCS
AA
AK
KK
DGAT1: ns
DGAT1 x Lact: ns
Days in Milk

Figure 1. Estimates for DGAT1 by lactation stage interaction for parity 1 cows for milk-, fat- and protein yield (in Kg), fat%, protein%, lactose%, Somatic Cell Score (SCS) and total Energy output in milk (TEM in MJ) and the significance of the effect of DGAT1 and DGAT1 by stage of lactation interaction (DGAT1*Lact)

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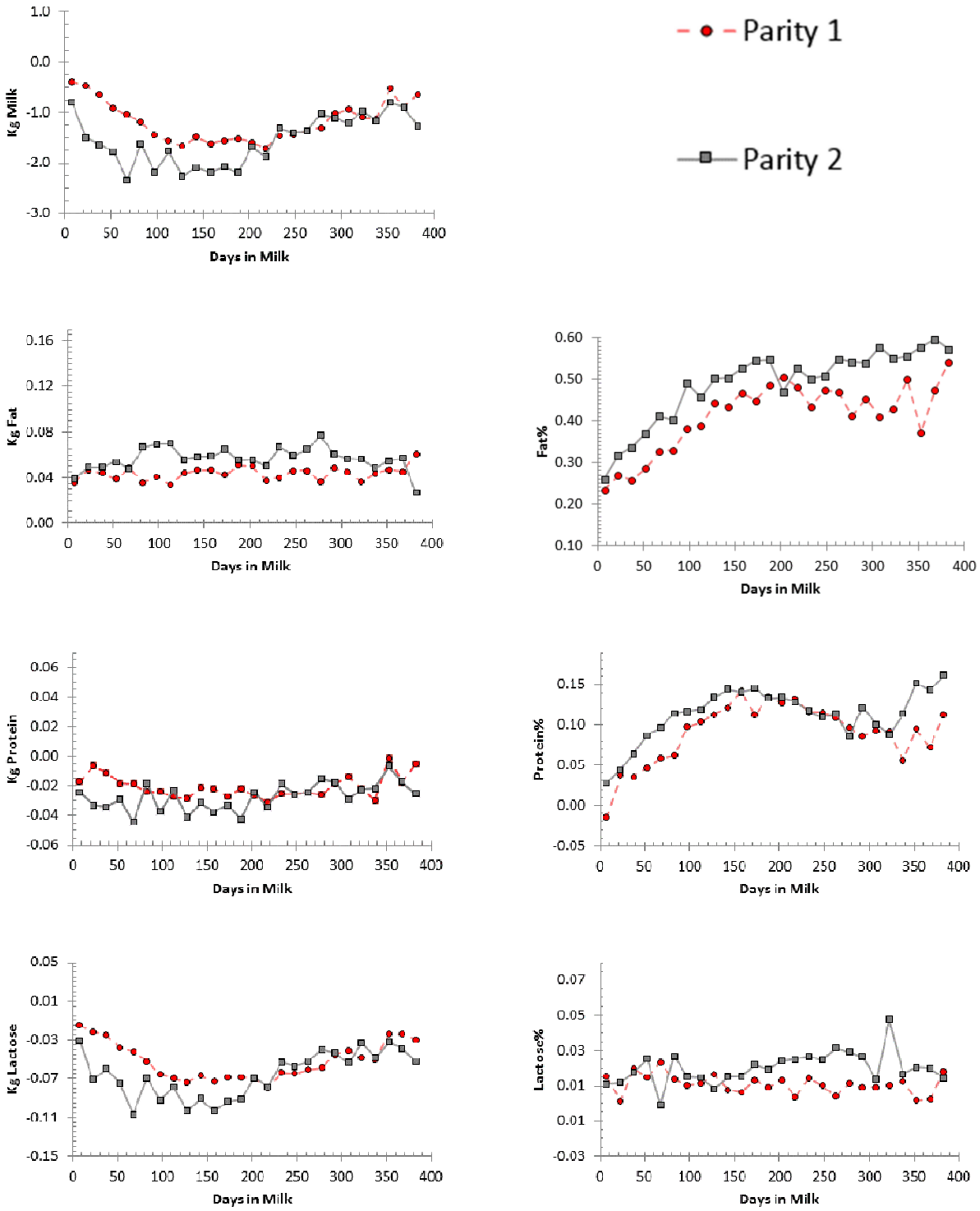

488 **Figure 2.** The additive effect $\left(\frac{KK-AA}{2}\right)$ of the DGAT1 polymorphism on test-day milk production

489 traits for different lactation stages in parity 1 and 2.