Phenotypic and genetic effects of pregnancy on milk production traits in Holstein-Friesian cattle

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ABSTRACT

Pregnancy is a prerequisite for the initiation of lactation and for maintaining the milk production cycle. Pregnancy affects milk production and therefore should be accounted for in the genetic evaluation. Furthermore, there might be genetic differences in pregnancy effects on milk composition. The objective of this study was to estimate phenotypic and genetic effects of pregnancy on milk production traits. For this purpose, test-day records and conception dates of 1,359 first-parity Holstein-Friesian cows were analyzed. Significant effects of pregnancy on all milk production traits were detected except somatic cell score (e.g., the cumulative effects of pregnancy on milk yield were -247 kg). The pregnancy effects on milk yield, lactose yield, protein yield, fat yield, and fat content were small during early gestation (<150 d) and substantially increased in late gestation. The effects of pregnancy on milk protein yield were relatively stronger than those on fat yield. The effects of pregnancy on milk production traits differed for *DGAT1* genotypes. Milk yield, lactose yield, protein yield, and fat yield of *DGAT1* AA cows were more affected by pregnancy than that of *DGAT1* KK cows (e.g., the cumulative effects of pregnancy on milk yield were negligible for *DGAT1* KK cows and were -443 kg for *DGAT1* AA cows). These results suggest that *DGAT1* KK cows may be more suitable for shortening or omitting the dry period than *DGAT1* AA cows.

Key words: pregnancy effect, *DGAT1* by pregnancy stage interaction, nutrient allocation during pregnancy

INTRODUCTION

Pregnancy is a prerequisite for the initiation of lactation and for maintaining the milk production cycle. Pregnancy also affects milk production traits (Ragsdale et al., 1924; Erb et al., 1952; Coulon et al., 1995). It has been estimated that as a consequence of pregnancy,

305-d milk yield of a first-parity Holstein-Friesian cow is reduced by 207 kg as compared with a nonpregnant cow (Olori et al., 1997). Effects of pregnancy on milk yield and other milk production traits are relevant to the genetic evaluation of dairy cattle, and in several countries genetic evaluation accounts for pregnancy effects. Otherwise cows with better fertility, which are more likely to be pregnant, would be penalized in their breeding value for milk production traits (Olori et al., 1997; Bohmanova et al., 2009; Loker et al., 2009).

Pregnancy effects on milk production traits strongly depend upon the stage of pregnancy; effects of pregnancy on milk yield are small during early pregnancy (<150 d in gestation), after which effects start to increase nonlinearly (Olori et al., 1997; Bohmanova et al., 2009; Loker et al., 2009). Pregnancy effects in late gestation are relevant for assessing the impact of shortening or omitting the dry-off period; shortening the dry-off period will result in additional milk production that might compensate for decreased milk production in the next lactation (Kok et al., 2016). As pregnancy affects milk yield and composition, studies have been performed to investigate possibilities to predict pregnancy status based on milk infrared spectra (Toledo-Alvarado et al., 2018; Delhez et al., 2020). Pregnancy might also affect the genetic background of milk production traits. It has been found that effects of some genes related to milk production traits change during lactation and it has been suggested that changes during late lactation might be related to pregnancy (Lu and Bovenhuis, 2019; Lu et al., 2020). However, so far no studies have investigated if there are genetic differences in pregnancy effects on milk production traits. The objective of this study was to estimate the effects of pregnancy on milk production traits and to investigate if these effects differ between cows with different genotypes.

MATERIALS AND METHODS

Data

The cows for this study were part of the Dutch Milk Genomics Initiative and details about data collection

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and data structure can be found in Stoop et al. (2007). In brief, all cows were at least 87.5% Holstein-Friesian and were housed on 398 commercial herds with at least 3 cows per herd. For the current study, a subset of cows was selected from the original data set and selection was based on the possibilities to accurately predict date of conception. Cows were only selected if the calving date for the second parity and insemination records in first-parity were available. For those cows, date of conception was estimated based on the calving date for the second parity minus an average gestation length of 279 d. Cows were only included in our analyses if the last registered insemination record in the first parity was within a 30-d interval of the estimated conception date (± 15 d). Of the available 1,800 first-parity cows, 1,359 cows met these criteria and were used for further analyses.

Test-day milk production records of 1,359 first-parity cows were available including milk yield, lactose yield, lactose content, protein yield, protein content, fat yield, fat content, and SCC. The total number of test-day records was 14,505 with slight differences between traits and each cow on average had 10.7 test-day records. All test-day records were obtained from routine milk recordings. Lactose, fat, and protein content were determined by infrared spectroscopy (MilkoScan FT 6000, Foss Electric, Hillerød, Denmark) at the milk control station laboratory (Qlip, Zutphen, the Netherlands). Lactose, fat, and protein yield were calculated by multiplying respective content by milk yield. Somatic cell count was determined using the Fossomatic 5000 (Foss Electric) and analyzed as SCS, i.e., $\log_e(\text{SCC})$.

Pregnancy Effects on Milk Production Traits

Pregnancy effects on milk production traits were estimated using the following model:

$$y_{jklmnopq} = \mu + b_1 \cdot afc_{jklmnopq} + season_j + scode_k + lact_l + PS_n + HTD_o + animal_p + pe_q + e_{jklmnopq} \quad [1]$$

where $y_{jklmnopq}$ is the test-day milk production traits; μ is the overall mean; $afc_{jklmnopq}$ is a covariate describing the effect of age at first calving and b_1 is the regression coefficient; $season_j$ is the fixed effect of calving season (4 classes: August to October, November to January, February to April, and May to July); $scode_k$ is the fixed effect accounting for possible differences in genetic level between proven bulls (638 daughters), test bulls (586 daughters), and other proven bulls (135 daughters); $lact_l$ is the fixed effect of lactation stage (26 stages of 15 d each); PS_n is the fixed effect of pregnancy stage (stage 0 indicating the period before conception and 8 preg-

nancy stages of 30 d each); HTD_o is the random effect of herd-test-day, which is assumed to be distributed as $N(\mathbf{0}, \mathbf{I}\sigma_{HTD}^2)$, where $\mathbf{I}$ is an identity matrix and σ_{HTD}^2 is the herd-test-day variance; $animal_p$ is the random additive genetic effect of the individual and assumed to be distributed as $N(\mathbf{0}, \mathbf{A}\sigma_a^2)$, where $\mathbf{A}$ is the additive genetic relationships matrix and σ_a^2 is the additive genetic variance; pe_q is the permanent environmental effect and assumed to be distributed as $N(\mathbf{0}, \mathbf{I}\sigma_{pe}^2)$, where $\mathbf{I}$ is an identity matrix and σ_{pe}^2 is the permanent environmental variance; and $e_{jklmnopq}$ is the random residual and assumed to be distributed as $N(\mathbf{0}, \mathbf{I}\sigma_e^2)$, where $\mathbf{I}$ is an identity matrix and σ_e^2 is the residual variance. The additive genetic relationship matrix ($\mathbf{A}$) was constructed based on 14,062 animals (traced back 5 generations). The pedigree of the cows was provided by the Dutch herd book (CRV, Arnhem, the Netherlands). The model also included the effect of $scode_k$. Proven bulls are not a random sample, but they are selected, and this might affect the estimated additive genetic variance. Possible differences in genetic level between groups of bulls are accounted for by the effect of $scode_k$.

Lactation was truncated at 390 d instead of 305 d, resulting in 26 lactation stage classes of 15 d each. Truncation at 390 d increased the number of test-day records in late lactation and the number of test-day records for late pregnancy stages. Test-day records before the conception date were assigned to pregnancy stage 0 and from the conception date onward 8 pregnancy stages of 30 d each were defined. After 240 d of pregnancy, most cows were dried off and the remaining 26 test-day records were excluded from the analysis.

The significance of PS_n on milk production traits was tested using the Wald F -test statistic. Differences between estimated effects for pregnancy stage 0 and other pregnancy stages were tested using a t -test. If the P -value was < 0.001 , the effect of that pregnancy stage on a milk production trait was considered significantly different from the effect of pregnancy stage 0.

Genetic Differences in Pregnancy Effects

In our previous studies we identified several QTL whose effects on milk production traits changed during lactation (Lu and Bovenhuis, 2019; Lu et al., 2020). Some of the changes in genetic effects occur in early lactation and might be related to negative energy balance. Others occur in late lactation and we hypothesized that these changes might be related to pregnancy. We

tested this hypothesis for the following SNP by trait combinations: rs29011303, rs43193272, rs41591350, and rs109651365 for protein content; rs523413537 for milk yield, lactose yield, protein content, and fat content; and rs41578697 for lactose content. The SNP genotypes were available based on a 50K SNP panel as described in Schopen et al. (2011). The SNP rs523413537 is 1 of the 2 SNP responsible for the diacylglycerol O-acyltransferase 1 (*DGAT1*) K232A polymorphism and therefore will be referred to as *DGAT1* in the remaining part of this paper. The frequency of the *DGAT1* K allele in the current population is 0.40. To investigate the hypothesis that changes in SNP effects are related to pregnancy, we used the following model:

$$y_{ijklmnopq} = \mu + b_1 \cdot afc_{ijklmnop} + season_j + scode_k + lact_l + SNP_m + (SNP \times lact)_{lm} + PS_n + (SNP \times PS)_{mn} + HTD_o + animal_p + pe_q + e_{ijklmnopq} \quad [2]$$

where SNP_m is the fixed effect of the SNP genotype (3 genotype classes); $(SNP \times lact)_{lm}$ is the genotype by lactation stage interaction, which allows SNP effects to change during lactation; and $(SNP \times PS)_{mn}$ is the genotype by pregnancy stage interaction, which allows SNP effects to change during pregnancy. Other model terms are as described for model [1].

The significance of $(SNP \times PS)_{mn}$ in model [2] was tested using the Wald *F*-test statistic. The test statistic of the interaction term is inflated (Lu and Bovenhuis, 2019) and therefore the significance of the $(SNP \times PS)_{mn}$ model term was tested based on 1,000 permutations. In each permutation, *SNP* genotypes were randomly re-assigned to another animal. Subsequently, the permuted data were re-analyzed and the Wald *F*-test statistics of the $(SNP \times PS)_{mn}$ term were stored. These Wald *F*-test statistics were used to determine the 5% significance threshold level of the $(SNP \times PS)_{mn}$ interaction term. In case $(SNP \times PS)_{mn}$ was significant, a *t*-test was used to test if genotypes significantly differed within a pregnancy stage. If the *P*-value was smaller than 0.001, the SNP effect within that pregnancy stage was considered significant. All the analysis were performed in Asreml (Gilmour et al., 2006).

RESULTS AND DISCUSSION

Pregnancy Effects on Milk Production Traits

The number of records, mean, and standard deviation for the test-day milk production traits, days open, and age at first calving for 1,359 first-parity Holstein-Friesian cows are in Table 1. On average, cows were pregnant at 130 d after calving, but there were substan-

Table 1. Descriptive statistics of test-day milk production traits, days open, and age of first calving for 1,359 first-parity Holstein-Friesian cows

Trait	Number	Mean	SD
Milk yield (kg/d)	14,505	24.63	5.30
Lactose yield (kg/d)	14,341	1.15	0.25
Lactose content (%)	14,341	4.65	0.14
Protein yield (kg/d)	14,469	0.86	0.17
Protein content (%)	14,469	3.51	0.31
Fat yield (kg/d)	14,460	1.06	0.22
Fat content (%)	14,460	4.37	0.63
SCS	13,555	4.14	1.02
Days open (d)	1,359	130	77
Age at first calving (yr)	1,359	2.13	0.16

tial differences; the days open range from 55 d (5th percentile) to 284 d (95th percentile) in lactation. These large differences in conception date allow disentangling the effects of pregnancy and lactation stage. Average age at first calving was 2.13 yr and ranged from 1.80 to 2.98 yr.

Pregnancy had significant effects on all milk production traits except SCS (model [1]). Figure 1 shows the estimated effects of the different pregnancy stages on milk production traits. In Figure 1 traits were expressed in phenotypic standard deviations to make effect sizes comparable across traits. Cumulative pregnancy effects were calculated as the cumulative differences between pregnant and nonpregnant cows (pregnancy stage 0). Cumulative effects of pregnancy over pregnancy stages 1 through 8, that is, $\sum_{n=0}^{n=7} 0.5 \cdot 30 \cdot (PS_n + PS_{n+1})$, on milk yield were -247 kg, on lactose yield -11.6 kg, on protein yield -10.4 kg, and on fat yield -8.2 kg. When expressed in phenotypic standard deviations, pregnancy effects on protein yield were more pronounced than those on fat yield (Figure 1). The effects of pregnancy on lactose content and protein content were small. Pregnancy effects on milk yield, lactose yield, protein yield, fat yield, and fat content were relatively small in early pregnancy and became more important in late pregnancy [e.g., pregnancy effects on milk yield from stage 5 (d 150) onward accounted for 88% of the total cumulative effects of pregnancy on milk yield].

For first-parity Holstein-Friesian cows, Olori et al. (1997) estimated pregnancy effects on 305-d milk production of -207 kg of milk, -10.7 kg of lactose, -8.7 kg of protein, and -8.1 kg of fat. For Canadian Ayrshire, Loker et al. (2009) estimated pregnancy effects of -222 kg of milk, -10.0 kg of protein, and -7.3 kg of fat. The latter estimates were based on the first-, second-, and third-parity test-day records with DIM ≥ 5 and ≤ 365 (Loker et al., 2009). These pregnancy effects on milk production traits differ slightly from the estimates in the current study. Like the current study,

Figure 1. Estimated effects ($\pm$ SE) of pregnancy on milk production traits for different pregnancy stages. The y-axis is scaled by the phenotypic standard deviation (in parentheses) of the corresponding traits. The dashed line indicates the effect of pregnancy stage 0 (nonpregnant). A significant ($P < 0.001$) difference between effects of a pregnancy stage and pregnancy stage 0 based on a t -test is indicated by *.

other studies also reported more pronounced effects of pregnancy on protein yield than on fat yield (Olori et al., 1997; Bohmanova et al., 2009; Loker et al., 2009). Milk lactose is the major component for milk osmosis and does not show much variation (Fox et al., 2015; Costa et al., 2019). This explains why milk lactose content is not strongly affected by pregnancy. The effects of pregnancy on protein content are also small, which is due to relatively similar pregnancy effects on protein yield and milk yield. Consequently, the effect of pregnancy on protein content (i.e., the ratio of protein yield

over milk yield) is small. Similar to the current study, other studies also reported that pregnancy effects on milk yield, lactose yield, protein yield, fat yield, and fat content mainly occur during late gestation (e.g., Olori et al., 1997; Bohmanova et al., 2009; Penasa et al., 2016).

Pregnancy effects on milk production traits are likely due to nutrient partitioning during gestation (Bauman and Currie, 1980; Bell, 1995). The availability and allocation of nutrients during gestation involves feed intake, nutrient absorption, milk production, maintenance,

Table 2. The *P*-values for SNP by pregnancy stage interaction of a selected set of SNP¹ whose effects change during late lactation

SNP	BTA	Position ² (bp)	Trait	<i>P</i> -value ³ (SNP × PS)
rs29011303	3	93,216,176	Protein content	0.54
rs43193272	9	85,934,554	Protein content	0.89
rs41591350	10	48,721,829	Protein content	0.66
rs523413537	14	445,087	Milk yield	<0.001
rs523413537	14	445,087	Lactose yield	<0.001
rs523413537	14	445,087	Protein content	0.004
rs523413537	14	445,087	Fat content	0.43
rs41578697	19	57,745,840	Lactose content	0.73
rs109651365	27	37,915,598	Protein content	0.78

¹SNP were from studies by Lu and Bovenhuis (2019) and Lu et al. (2020). SNP rs523413537 is 1 of the 2 SNP responsible for the *DGAT1* K232A polymorphism.

²SNP position based on Btau 4.0.

³The *P*-values for genotype by pregnancy stage interaction (SNP × PS) were based on 1,000 permutations.

body reserve, and fetus development. In addition, for primiparous cows part of their body growth takes place during the first lactation and therefore growth is an additional nutrient demanding process. Pregnancy effects on milk production traits might therefore be due to several factors, one of them being that nutrients partitioned to fetus development are no longer available for milk production. Stronger effects of pregnancy on protein yield than fat yield might be related to the requirements of the fetus; protein requirement of the fetus is nearly twice that of the fat requirement (Prior and Laster, 1979). Increased pregnancy effects toward late gestation on milk yield, lactose yield, protein yield, and fat yield is also in line with the nutrient requirements of the fetus; growth of the fetus increases exponentially from d 160 in gestation onward. In the last 2 mo of pregnancy (i.e., the period during which most cows are dried off), the fetus acquires around 60% of its birth weight (Prior and Laster, 1979; Bauman and Currie, 1980; Bell et al., 1995; Krog et al., 2018). For management purposes, it is assumed that the energy required for the development of the fetus is nonsignificant during early gestation and extra energy for gestation is only considered from 190 d in gestation onward (NRC, 2001).

Genetic Differences in Pregnancy Effects

In previous studies we identified chromosomal regions whose effects change during late lactation and hypothesized that these changes might be related to pregnancy (Lu and Bovenhuis, 2019; Lu et al., 2020). The lead SNP of the chromosomal regions previously identified for specific SNP by trait combinations are shown in Table 2, and it was tested if changes in these SNP effects can be attributed to pregnancy. The SNP rs523413537 on BTA14 (*DGAT1*) showed significant

SNP by pregnancy stage interactions (SNP × PS) for milk yield, lactose yield, and protein content. For the other 6 SNP by trait combinations, we did not find evidence that changes in SNP effects during lactation can be attributed to pregnancy. In our previous study, changing *DGAT1* effects during late lactation on milk yield, lactose yield, protein content, and fat content were detected based on stringent genome-wide significance thresholds (Lu and Bovenhuis, 2019; Lu et al., 2020), which reduces detection power. However, it is well documented that the *DGAT1* polymorphism has major effects on several milk production traits (e.g., Grisart et al., 2002; Bovenhuis et al., 2016). Therefore, the *DGAT1* by pregnancy stage interaction for other milk production traits (protein yield, fat yield, lactose content, and SCS) were also tested using model [2], and significant interactions were detected for protein yield ($P = 0.008$) and fat yield ($P = 0.009$).

The estimated effects of *DGAT1* during pregnancy on milk yield, lactose yield, protein yield, fat yield, and protein content are shown in Figure 2. The estimated effects for SNP by pregnancy stage interaction were from model [2] without the main effects of SNP and pregnancy stage. Figure 2 shows that milk yield, lactose yield, protein yield, and fat yield of *DGAT1* AA cows are more affected by pregnancy than those of *DGAT1* KK cows. For *DGAT1* KK cows, pregnancy reduced milk yield, lactose yield, protein yield, and fat yield from d 180 in pregnancy onward, for AK cows reduction in milk yield, lactose yield, protein yield, and fat yield started around d 120 in pregnancy, and for AA cows pregnancy affected milk yield, lactose yield, protein yield, and fat yield shortly after conception. Figure 2 also shows that differences in protein content for cows with different *DGAT1* genotypes are relatively constant throughout gestation but differences between genotypes tend to decrease toward late gestation. Estimates

Figure 2. Effects ($\pm$ SE) of *DGAT1* genotypes on milk yield, lactose yield, protein yield, fat yield, and protein content during pregnancy. The effects were estimates for SNP by pregnancy stage interaction from a model without main effects of SNP and pregnancy stage. The y-axis is scaled by the phenotypic standard deviation (in parentheses) of the corresponding trait. The dashed line indicates the effects of pregnancy stage 0 (nonpregnant), separately for each *DGAT1* genotype. * indicates a significant ($P < 0.001$) difference between any 2 SNP genotype classes in that specific pregnancy stage based on a t -test.

for cumulative pregnancy effects on milk yield, lactose yield, protein yield, and fat yield over pregnancy stages 1 through 8 for cows with different *DGAT1* genotypes are shown in Table 3. Effects differed substantially for *DGAT1* genotypes (e.g., pregnancy effects were -443 kg of milk for AA cows, -233 kg for AK cows, and 26 kg for KK cows). For KK cows the estimated effects of pregnancy from d 1 through d 180 were small and positive, and cumulative effects over pregnancy from d 180 to 240 were -36.5 kg of milk.

Differences in pregnancy effects between cows with *DGAT1* genotypes might be due to differences in fertil-

ity; cows with a higher number of days open might show smaller effects of pregnancy on milk production. Therefore, we also compared days open for cows with different *DGAT1* genotypes; for AA cows this was 127 d, for AK cows it was 136 d, and for KK cows it was 123 d. These differences were not significant and cannot explain the observed differences between *DGAT1* genotypes in pregnancy effects on milk production traits. Pregnancy effects on milk production might be due to changes in the allocation of nutrients during gestation that involves feed intake, milk synthesis, maintenance, body reserve, fetus development, and growth for pri-

Table 3. Cumulative effects (kg) of pregnancy on first-parity milk yield, lactose yield, protein yield, and fat yield for cows with different *DGAT1* genotypes

<i>DGAT1</i>	Milk yield	Lactose yield	Protein yield	Fat yield
AA	-443	-20.6	-16.3	-13.5
AK	-233	-11.2	-9.7	-9.4
KK	26	1.0	-2.2	4.6

miparous cows (Bauman and Currie, 1980; Bell, 1995). Milk production of KK cows was less affected by pregnancy than that of AA cows, which might indicate that KK cows partition fewer nutrients from milk synthesis to fetal growth. If so, the birth weight of calves from KK cows should be lower than calves from AA cows and consequently KK cows would have less calving problems than AA cows. However, there is currently no evidence that KK cows produce calves with lower birth weight (Cole et al., 2014) and have better calving ease than AA cows (Berry et al., 2010). There also might be alternative explanations for the observed effects of *DGAT1* genotypes (e.g., differences in feed intake, body reserve, and age of first calving). However, Banos et al. (2008) did not detect significant effects of *DGAT1* genotypes on feed intake and body reserve. Furthermore, in the current population age at first calving was not significantly affected by the *DGAT1* polymorphism (results not shown). Therefore, the physiological background of observed differences in pregnancy effects of *DGAT1* genotypes on milk production traits requires further investigation.

Conventionally, dairy cows are dried off in late gestation and in negative energy balance after calving due to the peak production and constrained feed intake (e.g., Collard et al., 2000; de Vries and Veerkamp, 2000). Alternative management strategies such as shortening or omitting the dry-off period have been proposed to reduce peak production after calving (e.g., Chen et al., 2015; Kok et al., 2017; van Hoeij et al., 2017). This management strategy results in additional milk yield during late gestation and decreased milk yield in the next lactation. Kok et al. (2016) suggested that the suitability of this management strategy should be assessed with consideration of milk yield during the last 60 d in gestation (i.e., the conventional dry-off period). Our study showed that milk yield during late gestation was affected by pregnancy and effects of pregnancy differ between cows with different *DGAT1* genotypes. This suggests that the suitability of cows for shortening or omitting the dry-off period might depend upon their *DGAT1* genotype. Further research is needed to study how cows with different *DGAT1* genotypes respond to shortening or omitting the dry-off period.

It has been suggested that after 150 d in gestation pregnancy status can be predicted based on milk infrared spectra (Lainé et al., 2017; Toledo-Alvarado et al., 2018; Delhez et al., 2020). Such information might be used as a complementary tool to detect fetal abortion (Delhez et al., 2020). The current study shows that there are significant differences in pregnancy effects on milk production traits between cows with different *DGAT1* genotypes. Milk production traits of *DGAT1* KK cows are hardly affected by pregnancy. Therefore, accuracy

of predicting pregnancy based on milk infrared spectra might be poor. Effects of pregnancy for *DGAT1* AA cows are stronger and start earlier in pregnancy, which might result in higher prediction accuracies. Wang and Bovenhuis (2020) demonstrated that combining milk infrared spectra with genotypic information can improve prediction of milk fat composition. Therefore, accounting for genetic differences in pregnancy effects might improve prediction of pregnancy by milk infrared spectra.

CONCLUSIONS

Significant effects of pregnancy on milk production traits were detected. Pregnancy effects on milk yield, lactose yield, protein yield, fat yield, and fat content were mainly observed during late pregnancy (>d 150). Pregnancy effects on protein yield were higher than those on fat yield. Interestingly, pregnancy effects on milk yield, lactose yield, protein yield, fat yield, and protein content differed considerably between cows with different *DGAT1* genotypes.

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