Genome-wide scan to detect quantitative trait loci for milk urea nitrogen in Dutch Holstein-Friesian cows

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ABSTRACT

Studies have reported genetic variation in milk urea nitrogen (MUN) between cows, suggesting genetic differences in nitrogen efficiency between cows. In this paper, the results of a genome-wide scan to identify quantitative trait loci (QTL) that contribute to genetic variation in MUN and MUN yield are presented. Two to 3 morning milk samples were taken from 1,926 cows, resulting in 5,502 test-day records. Test-day records were corrected for systematic environmental effects using a repeatability animal model. Averages of corrected phenotypes of 849 cows, belonging to 7 sire families, were used in an across-family multimarker regression approach to detect QTL. Animals were successfully genotyped for 1,341 single nucleotide polymorphisms. The QTL analysis resulted in 4 chromosomal regions with suggestive QTL: *Bos taurus* autosomes (BTA) 1, 6, 21, and 23. On BTA 1, 2 suggestive QTL affecting MUN were detected at 60 and 140 cM. On BTA 6, 1 suggestive QTL affecting both MUN and MUN yield was detected at 103 cM. On BTA 21, 1 suggestive QTL affecting MUN yield was detected at 83 cM. On BTA 23, 1 suggestive QTL affecting MUN was detected at 54 cM. Quantitative trait loci for MUN and MUN yield were suggestive and each explained between 2 and 3% of the phenotypic variance.

Key words: genome-wide scan, milk urea nitrogen, quantitative trait loci

INTRODUCTION

In the Netherlands, fertilization of agricultural land with N from animal manure is limited by the Dutch manure policy, implemented in 2006 to reduce pollution of ground and surface water (LNV, 2006). Many Dutch dairy farms produce more N in manure than they are allowed to use on their own land (Sebek et al., 2007). As a consequence, the manure surplus of dairy farms has to be disposed of.

The amount of N excreted by dairy cows depends on the excess of ammonia in the rumen. Degradation of CP by rumen bacteria and protozoa in dairy cattle results in ammonia. Most rumen bacteria use ammonia as the main source of N for microbial protein production (Bryant, 1970). Unused ammonia is converted to urea in the liver or kidney and absorbed into the bloodstream of the cow (Nolan and Dobos, 2005). Urea in the bloodstream is recycled to the rumen, secreted in milk or excreted through urine. Recycling of urea occurs by diffusion of urea from the blood into saliva or directly across the rumen wall, reentering the digestive tract (Huntington and Archibeque, 2000; Nolan and Dobos, 2005). Urea in the bloodstream easily diffuses across mammary tissues, resulting in equilibrium between urea levels in blood and milk (Gustafsson and Palmquist, 1993); the urea in milk is secreted as MUN. Urea is filtered from the blood by the kidneys and excreted through urine (Butler, 1998), ending up in the (semiliquid) manure of cows. Milk urea N is highly correlated to N excretion in urine of dairy cows (Jonker et al., 1998) and is very easy to record in contrast to BUN or N in urine. Therefore, MUN is used in the Dutch manure policy to estimate the amount of N in the manure of dairy cows (LNV, 2008).

Reducing MUN reduces the estimated amount of N in manure so that the amount of manure that is allowed to use for fertilization increases, which can lead to a reduction of manure disposal cost. In 2008, 6.25 million tonnes of cattle manure was disposed in the Netherlands (LNV Loket, 2009). Disposing manure from farms costs around €10–15 per tonne at this moment, but strongly depends on market supply (Luesink et al., 2009). Reducing MUN is economically favorable for dairy farmers and leads to a reduction of environmental pollution.

Milk urea N reflects the N efficiency of dairy cows. Utilization of dietary N by ruminants is most efficient when there is enough dietary energy available to convert all ammonia to microbial protein so that little ammonia will be converted to urea and excreted (Lewis, 1957; Bryant, 1970). Protein in dairy cow diets is the main determinant of N excretion of dairy cows, but several studies have reported genetic variation in

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MUN between cows, suggesting genetic differences in N efficiency. Heritability estimates for MUN ranged between 0.14 and 0.44 (Wood et al., 2003; Mitchell et al., 2005; De Roos and De Jong, 2006; Stoop et al., 2007). Gaining knowledge about the genetic basis underlying MUN will increase the understanding of N utilization of dairy cows. The first steps toward this knowledge have been made by showing that there is genetic variation in MUN; a second step would be the identification of QTL affecting MUN. Quantitative trait loci have been mapped for many milk composition traits in several cattle populations (see review by Khatkar et al., 2004). To our knowledge, no QTL have been reported for MUN. The aim of this study was therefore to perform a genome-wide scan to detect QTL affecting MUN concentration and MUN yield.

MATERIALS AND METHODS

Phenotypes

This study is part of the Dutch Milk Genomics Initiative, which studies the genetic aspects of detailed milk composition. For this study, phenotypic data were recorded from 1,926 first-lactation cows coming from 398 herds throughout the Netherlands. The cooperative cattle improvement organization (CVR, Arnhem, the Netherlands) provided the pedigree of all animals. The pedigree included 26,300 animals and went back to 1928. Each cow was at least 87.5% Holstein-Friesian. Three sire groups could be distinguished: 5 proven sires with 884 daughters, 50 test-sires with 856 daughters, and 15 other proven sires with 186 daughters. The latter sire group ensured at least 3 sampled cows per herd.

The cows were milked twice daily and were between 41 and 335 DIM. Morning milk samples were taken on 3 test-days between February and June 2005. Some cows were removed from farms during this period, or some of the milk samples were missing because of other reasons; therefore, not all cows had 3 milk samples. Animals with only 1 milk sample were excluded from the data set. Thus, the 1,926 cows had 2 or 3 test-day records, resulting in 5,502 test-day records in total. During the winter period almost all cows were kept indoors and fed silage; during the summer period about half of the cows grazed on pasture during part of the day.

Milk urea N was determined by infrared spectroscopy using a Fourier transformed interferogram (MilkoScan FT6000, Foss Electric, Hillerød, Denmark) at the laboratory of the Milk Control Station (Zutphen, the Netherlands). Milk urea N yield was calculated by multiplying MUN with morning milk yield, which was obtained from CRV, the official milk recording organization. More detailed information about the milk samples can be found in Stoop et al. (2007).

The phenotypes used for the QTL analysis were pre-corrected for 5 systematic environmental effects: DIM, age at first calving, season of calving, herd-test-day, and permanent environment of the cow. In total, 5,502 test-day records of 1,926 cows were used to estimate systematic environmental effects with a repeatability animal model in ASReml (Gilmour et al., 2006), identical to the model described by Stoop et al. (2007). The average precorrected test-day records of the cows were used for the QTL analysis. Cows had at least 2 test-day records; thus, the adjusted phenotypes were averages of 2 or 3 precorrected test-day records.

Genotypes

Semen samples were taken from the sires and blood samples from their daughters to collect DNA. Only the 5 large families from the proven sires and 2 additional small families were genotyped. Five proven sires, 2 test sires, and their 849 daughters were successfully genotyped for 1,341 SNP using the GoldenGate assay (Illumina, San Diego, CA). The genetic map based on these 1,341 SNP covered 2,829 cM on the Haldane scale. The information content of the SNP was calculated as the variance of the probability that, at a specific SNP position, a daughter inherited a given allele (allele 1) from its sire (Spelman et al., 1996). Further details about the selection of the SNP, linkage maps, and information content are given by Schopen et al. (2009).

QTL Analysis

Adjusted phenotypes of 849 cows, which were successfully genotyped, were used for the QTL analysis. To get more accurate estimates of the systematic environmental effects, the test-day records of all 1,926 cows (5,502 test-day records) were used. Only 849 cows, however, were genotyped, so only their average precorrected test-day records were included as adjusted phenotypes in the QTL analysis. The number of daughters per proven sire was 193, 179, 170, 166, and 91, and number per test sire was 29 and 21.

Quantitative trait loci analysis was performed using a multimarker regression approach for half-sib designs, according to Knott et al. (1996). The regression was based on interval mapping across families. The most likely position for a QTL was the position with the highest test statistic from the across-family analysis. The QTL analysis was performed for each trait on each chromosome using the model

$$Y_{ijk} = \mu + S_i + b_{ik}X_{ijk} + e_{ijk},$$

Table 1. Mean and coefficient of variation for MUN and MUN yield measured on 2 or 3 morning milk samples of 1,926 primiparous Holstein-Friesian cows

Trait	n	Mean	CV (%)
MUN (mg/100 g)	5,498	20.38	33
MUN yield (g)	5,218	2.65	40

where Y_{ijk} is the adjusted phenotype for daughter j of sire i at chromosomal position k ; μ is the overall mean; S_i is the effect of sire family i ; b_{ik} is the regression coefficient for sire i at chromosomal position k ; X_{ijk} is the probability of daughter j inheriting gamete 1 from sire i at position k ; and e_{ijk} is the random residual.

In case of significant or suggestive evidence for the presence of a QTL, allele substitution effects of individual sires were studied. Sires were considered to be heterozygous for a QTL if the allele substitution effect at the most likely position divided by its standard error was larger than 1.96 or smaller than -1.96 . This corresponds to the 5% significance threshold based on the t -distribution. The GLM procedure of SAS 9.1 (SAS Institute, Cary, NC) was used to estimate the percentage of phenotypic variance explained by a QTL across families. The difference in proportion of variance explained by the model (R^2), between the full model including the QTL ($b_{ik}X_{ijk}$) at the best position and the reduced model excluding this QTL, was used to estimate the proportion of variance explained by the QTL.

Significance Thresholds and Confidence Intervals

Threshold levels for chromosome-wise significance ($P_{\text{chromosome-wise}}$) per trait were calculated based on 10,000 permutations for each chromosome (Churchill and Doerge, 1994). Genome-wise P -values were obtained from the chromosome-wise P -values using a Bonferroni correction: $1 - (1 - P_{\text{chromosome-wise}})^{1/r}$, where r is the length of the chromosome divided by the length of the whole genome (de Koning et al., 1999). The permutation test takes multiple testing on a specific chromosome into account, whereas the Bonferroni correction takes mul-

tiples testing on the whole genome into account. Testing multiple traits was not taken into account, to obtain first preliminary results on QTL for MUN and MUN yield.

In this study, 2 significance thresholds were applied: suggestive linkage, which corresponds to the 5% chromosome-wise significance level, and significant linkage, which corresponds to the 5% genome-wise significance level (Lander and Kruglyak, 1995). To define the 95% confidence interval for a QTL location, 1,000 bootstraps were performed (Visscher et al., 1996).

RESULTS

The information content averaged 83% over the whole genome, with a minimum value of 54% and a maximum value of 97%. In regions on BTA 6, 9, 14, and 19, the information content was lower than 60%. Means and coefficients of variation for test-day morning MUN and MUN yield records are given in Table 1.

The QTL analysis resulted in 6 suggestive QTL for the 2 studied traits corresponding to 4 chromosomal locations (Table 2). Suggestive evidence for QTL affecting MUN was detected on BTA 1, 6, and 23. Suggestive evidence for QTL affecting MUN yield was detected on BTA 6 and 21. The graphical representation of the test statistic of the 2 traits on BTA 1, 6, 21, and 23 are given in Figures 1, 2, 3, and 4, respectively. The main results are described per chromosomal region.

On BTA 1, suggestive evidence for a QTL affecting MUN was detected at 60 cM (Figure 1). The QTL affecting MUN segregated in sire family 3 and 4. The allele substitution effect was about 0.4 phenotypic standard deviations (Table 3). There was also suggestive evidence for a second QTL affecting MUN on BTA 1 at 140 cM (Figure 1). This second QTL affecting MUN segregated in sire family 1 and 4. The allele substitution effect was about 0.45 phenotypic standard deviations (Table 3). However, the confidence interval of this second QTL at 140 cM is almost identical to the confidence interval of the QTL at 60 cM, which indicates that they possibly represent the same QTL.

Table 2. Location, 95% CI, significance levels, and the percentage of phenotypic variance explained by the QTL of the detected suggestive QTL for MUN and MUN yield¹

BTA	Trait	F-value	Position (cM)	95% CI (cM)	$P_{\text{chromosome-wise}}$	$P_{\text{genome-wise}}$	Var ² (%)
1	MUN	3.49	59.9	49–145	0.019	0.314	2.4
1	MUN	3.31	139.7	50–144	0.030	0.449	2.2
6	MUN	3.95	104.8	8–118	0.005	0.116	2.7
6	MUN yield	2.88	101.3	10–122	0.047	0.668	2.1
21	MUN yield	2.84	83.4	7–92	0.035	0.669	2.0
23	MUN	3.57	54.2	37–76	0.010	0.302	2.4

¹ $P_{\text{chromosome-wise}}$ = chromosome-wise P -value; $P_{\text{genome-wise}}$ = genome-wise P -value.

²Percentage of phenotypic variance explained by the suggestive QTL detected for MUN and MUN yield.

Figure 1. The test statistic of an across-family analysis for MUN (●) and MUN yield (+) on BTA 1. The dashed line represents the 95% chromosome-wise threshold for MUN. The continuous line shows the information content. The triangles on the x-axis indicate the marker positions.

On BTA 6, there was suggestive evidence for a QTL affecting both traits (Figure 2). For MUN and MUN yield, the most likely location of the QTL was at the telomeric end of BTA 6, around 103 cM. The QTL affecting both MUN and MUN yield segregated in sire family 1 and 3. The allele substitution effects indicated that the QTL affected MUN and MUN yield in the same direction, either increasing or decreasing both traits (Table 3).

On BTA 21, suggestive evidence for a QTL affecting MUN yield was detected at 83 cM (Figure 3). The QTL affecting MUN yield segregated only in sire family 4 (Table 3). The allele substitution effect was 0.55 phenotypic standard deviations (Table 3).

On BTA 23, suggestive evidence for QTL affecting MUN was detected at 54 cM (Figure 4). The QTL affecting MUN segregated in sire family 2 and 4. The allele substitution effects were around 0.4 phenotypic standard deviations (Table 3).

An overview of the percentage of phenotypic variance explained by each QTL is given in Table 2. The suggestive QTL explained between 2 and 3% of the phenotypic variance.

DISCUSSION

Four chromosomal regions have been detected affecting MUN and MUN yield. As far as is known, this study is the first to report QTL for MUN and MUN yield. Power calculations of the daughter design showed that the experiment had a good power (>0.80) to significantly detect QTL explaining 5 to 7.5% of the phenotypic variance when the heritability of a trait is 0.25. Heritability was 0.14 for MUN and 0.28 for MUN yield (Stoop et al., 2007). Most of the QTL reported in this study explained 2 to 3% of the phenotypic variance and were suggestive, which is in line with expectations based on the power calculations. The suggestive QTL

Figure 2. The test statistic of an across-family analysis for MUN (●) and MUN yield (+) on BTA 6. The dashed line represents the 95% chromosome-wise threshold for MUN yield. The threshold level varies per trait; in this case, the threshold was given for the trait with the lowest threshold. The continuous line shows the information content. The triangles on the x-axis indicate the marker positions.

need to be confirmed in other studies. The confidence intervals of the detected QTL were quite large; most of them were larger than 50 cM.

In the following section, the QTL detected in this study are compared with those reported in the literature. To be able to compare the regions, the position of the most nearby marker was used. For the marker positions, Genome Build 4.0 (Liu et al., 2009) was used.

On BTA 1, a suggestive QTL affecting MUN was detected at 60 cM. In the same region, Schulman et al.

(2004) detected a QTL for SCS, and Mosig et al. (2001) detected a QTL for protein percentage. Schopen et al. (2009) detected a suggestive QTL for the sum of casein at 57 cM using the same population and genetic map as in the present study. Mastitis infections are known to alter the milk protein composition. The casein fraction in milk from mastitic glands is lower than in milk from healthy glands, but the total amount of protein does not change because of a redistribution of milk N from casein to whey proteins (Haenlein et al., 1972; Ran-

Table 3. Phenotypic SD adjusted for systematic environmental effects (adj σ_p) and allele substitution effect, expressed in adjusted phenotypic SD, per sire for each QTL (SE in parentheses)

BTA	Trait	adj σ_p	Sire 1 (n = 193)	Sire 2 (n = 179)	Sire 3 (n = 170)	Sire 4 (n = 166)	Sire 5 (n = 91)	Sire 6 (n = 29)	Sire 7 (n = 21)
1	MUN _{60cM}	1.79	−0.15 (0.14)	−0.08 (0.14)	−0.44* (0.15)	0.40* (0.15)	0.39 (0.21)	0.17 (0.37)	0.82 (0.47)
1	MUN _{140cM}	1.79	0.34* (0.15)	0.10 (0.16)	−0.19 (0.16)	0.55* (0.16)	−0.25 (0.20)	0.37 (0.35)	0.61 (0.56)
6	MUN	1.79	0.35* (0.14)	−0.15 (0.15)	−0.69* (0.16)	−0.08 (0.16)	0.01 (0.21)	−0.25 (0.45)	0.59 (0.47)
6	MUN yield	309	0.45* (0.14)	0.05 (0.15)	−0.52* (0.17)	0.11 (0.16)	0.03 (0.24)	0.02 (0.45)	0.19 (0.51)
21	MUN yield	309	0.34 (0.19)	−0.12 (0.18)	−0.16 (0.16)	−0.55* (0.16)	−0.34 (0.22)	0.15 (0.51)	−0.83 (0.71)
23	MUN	1.79	−0.20 (0.14)	0.32* (0.15)	−0.29 (0.15)	0.49* (0.15)	0.37 (0.21)	0.37 (0.36)	−0.16 (0.40)

*Significant evidence that sire is heterozygous for the QTL according to a *t*-distribution with $\alpha = 0.05$.

Figure 3. The test statistic of an across-family analysis for MUN (●) and MUN yield (+) on BTA 21. The dashed line represents the 95% chromosome-wide threshold for MUN yield. The continuous line shows the information content. The triangles on the x-axis indicate the marker positions.

dolph et al., 1973; Urech et al., 1999). Limited studies have focused on the effect of mastitis on MUN or NPN, which consists largely of MUN. The genetic correlation between MUN and SCS was 0.85 according to Stoop et al. (2007), but was -0.19 according to Miglior et al. (2007). Ng-Kwai-Hang et al. (1985) found a small but significant increase in NPN between low and high somatic cell groups. Verdi et al. (1987), on the other hand, found that NPN was unaffected by SCC. Rajala-Schultz and Saville (2003) and Arunvipas et al. (2003), however, found that SCC on the individual cow level was negatively associated with MUN. The relationship between MUN and somatic cells remains unclear and deserves further research.

On BTA 1, a second suggestive QTL affecting MUN was detected at 140 cM. In this region, QTL affecting milk yield (Viitala et al., 2003; Daetwyler et al., 2008; Schulman et al., 2008), protein yield (Heyen et al., 1999; Daetwyler et al., 2008), days open (Schulman et al., 2008), fertility treatment (Schulman et al., 2008; Höglund et al., 2009), interval from calving until first

insemination (Höglund et al., 2009), and nonreturn rate (Ben Jemaa et al., 2008) have been reported. Quantitative trait loci studies that used the same population and genetic map detected QTL significantly affecting α_{S1} -casein (129 cM; Schopen et al., 2009) and suggestively affecting C18:0 (125 cM), C18 index (124 cM), and conjugated linoleic acid index (125 cM) in this region (Schennink et al., 2009). This region is thus associated with milk production, fertility, and protein and fat composition. Selection on milk production in the past has led to reduced fertility. Schulman et al. (2008) showed that their QTL affecting days open was linked to their QTL affecting milk yield and that their QTL effects were negatively correlated, supporting this negative correlation between milk production and fertility. Bobe et al. (2007) showed that selection for milk production had little effect on milk protein and milk fat composition. Only α_{S1} -casein levels significantly increased in cows with high predicted transmission ability for milk production. The fat composition was affected only by selection on milk production in the first month of lacta-

Figure 4. The test statistic of an across-family analysis for MUN (●) and MUN yield (+) on BTA 23. The dashed line represents the 95% chromosome-wise threshold for MUN. The continuous line shows the information content. The triangles on the x-axis indicate the marker positions.

tion, when cows are often in negative energy balance. In this period the proportion of C18:1 was higher in high-line cows compared with average-line cows. C18:1 is part of the C18 index and has a genetic correlation with C18:0 and conjugated linoleic acid of -0.41 and 0.54 , respectively (Stoop et al., 2008). The fact that the QTL affecting MUN was detected in the same region as others detected QTL for fertility traits can be explained by the negative effect of high MUN levels on fertility traits (Melendez et al., 2000; Rajala-Schultz et al., 2001; Guo et al., 2004). Mitchell et al. (2005) reported a genetic correlation between MUN and days open of 0.21 for first-lactation cows and an even stronger correlation (0.41) in second-lactation cows. König et al. (2008) reported a genetic correlation between MUN and interval from calving until first service of 0.29 . There is a direct effect of urea on fertility because BUN might interfere with embryo development because of its effect on uterine pH levels (Butler, 1998; Wathes et al., 2007). There is also an indirect effect of urea on fertility as a symptom of negative energy balance status

(Butler, 1998; Wathes et al., 2007). Early in lactation most high-producing cows enter a state of negative energy balance associated with many metabolic changes because they mobilize fat and muscle to support milk production. Diets high in CP demand energy from cows to convert the excess ammonia to into urea, which enhances a negative energy balance (Butler, 1998).

On BTA 6, a suggestive QTL affecting MUN and MUN yield was detected around 103 cM. Milk urea N and MUN yield have a genetic correlation of 0.77 (Stoop et al., 2007); therefore, it is likely to find QTL affecting both traits at approximately the same location, with allele substitution effects on both traits in agreement with the positive genetic correlation between both traits. Others reported QTL affecting the interval between calving and first insemination (Schrooten et al., 2000), SCS and clinical mastitis after second calving (Lund et al., 2008), and persistency of fat yield (Harder et al., 2006). Although there is no substantial evidence, the detected QTL in this region for milk production, fertility, mastitis, and MUN suggest that this

region is related to the metabolic pathways involved with a negative energy balance. The association among a negative energy balance, milk production, fertility, and MUN has been described above. A negative energy balance is also associated with mastitis occurrence because a negative energy balance impairs the udder defense mechanisms (Suriyasathaporn et al., 2000).

On BTA 21, a suggestive QTL affecting MUN yield was detected at 83 cM. In this region, Rodriguez-Zas et al. (2002) detected a QTL for SCS, and Heyen et al. (1999) detected a QTL affecting herd life. Miglior et al. (2006) showed that Canadian Holsteins with low MUN levels had a higher relative risk of culling. Also, high SCS has been associated with a reduced productive life. Genetic correlations between SCS and productive life have been reported around -0.30 (VanRaden et al., 2004; Tsuruta et al., 2005). Khatib et al. (2005a,b) reported that the uterine milk protein gene as well as the protease inhibitor gene had an effect on productive life. These genes are both located on BTA 21 within a 322-kbp genomic region of each other and near the QTL region. Two haplotypes of the protease inhibitor gene affected SCS as well. Unfortunately, the relationship between these genes and MUN was not studied, but they might be candidate genes for further research.

On BTA 23, a suggestive QTL affecting MUN was detected at 54 cM. In this region, QTL affecting SCS have been detected around 50 cM (Heyen et al., 1999; Ashwell et al., 2004; Daetwyler et al., 2008). Quantitative trait loci affecting protein yield at 69 and 42 cM (Bennewitz et al., 2003; Daetwyler et al., 2008) and milk yield at 55 cM (Bennewitz et al., 2003) were also detected within the region at BTA 23. Schopen et al. (2009) detected suggestive QTL affecting κ -casein and protein percentage at 40 and 43 cM, respectively, using the same population and genetic map.

Suggestive QTL affecting MUN and MUN yield were detected in regions where other studies have reported QTL affecting SCS, fertility, herd life, and milk production traits. The negative energy balance occurring in early lactation as a result of high production might be the underlying reason linking these QTL to each other. The energy metabolism of cows changes during lactation. Milk urea N levels also vary during lactation and are lowest in the first month of lactation (Rajala-Schultz and Saville, 2003; Stoop et al., 2007). Thus, different mechanisms might play a role for variation in N uptake and metabolism in different stages of lactation. Therefore, it might be worthwhile to analyze MUN levels per lactation stage as different traits. Given the large confidence intervals of the QTL positions and many pathways involved, there are too many genes that could be considered as candidate genes. Therefore further

research is necessary to narrow down the confidence intervals and to confirm these suggestive QTL.

In conclusion, the QTL analysis resulted in 4 chromosomal regions affecting MUN and MUN yield: BTA 1, 6, 21, and 23. The QTL for the MUN traits were suggestive and each explained between 2 and 3% of the phenotypic variance.

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