Effects of Genotype by Environment Interactions on Milk Yield, Energy Balance, and Protein Balance

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ABSTRACT

Increases in genetic merit for milk yield are associated with increases in mobilization of body reserves. This study assessed the effects of genotype by environment (G×E) interactions on milk yield and energy and protein balances. Heifers (n = 100) with high or low genetic merit for milk yield were milked 2 or 3 times a day and received rations of low or high caloric density. The management factors were selected to induce substantial differences in milk production levels and model different management strategies. The 2 × 2 × 2 factorial arrangement enables the assessment of the effects of genotype, environment, and G×E interactions. Mean daily energy-corrected milk production in the first 100 d in milk varied between 21.8 and 35.2 kg among the groups. The experimental factors affected milk production in the presumed direction. Ration was the most determinant factor on milk production. Effects of milking frequency and genetic merit were significant only in the groups that were fed rations with high caloric density. Signs for severe negative energy balances, protein balances, and low body condition scores, all of which may be indicative of health risks, were not concentrated in the highest producing cows. Feed caloric density and milking frequency had stronger effects on energy balances and protein balances, with unfavorable effects of low caloric density feed and an extra milking. This emphasizes the possible effect of mismanagement on animal health risks. High genetic merit cows had significantly lower postpartum body condition scores. Genotype × environment interactions existed, but more information is needed to determine if cows of different genetic merit for milk yield are differently at risk for disease under specific conditions. High milk production levels per se will increase allostatic load, but need not compromise the health status of relatively young cows. Ongoing one-sided selection for high yield may be combined with good animal health, but because high genetic merit for milk yield seems intrinsically connected to

the allocation of resources from maintenance toward milk, this puts increasing demands on farmers' time and management skills.

Key words: milk yield, energy balance, genotype by environment

INTRODUCTION

Milk production levels have increased around 2% per year (since 1985) in the United States and the Netherlands (Dillon et al., 2006), in part because of selective breeding. Increases in genetic merit for milk yield go together with increases in feed intake, but the latter does not fully compensate the extra energy demands during early lactation, resulting in a more negative energy balance (**NEB**) and increased mobilization of body reserves (van Arendonk et al., 1991). Severe NEB and the associated changes in metabolism play a central role in the genesis of production diseases (Goff and Horst, 1997; Drackley, 1999). In the present study, the role of energy balances in yield–health relationships is considered with emphasis on the roles of genotype, environment, and genotype × environment (**G×E**) interactions. The Danish Institute of Agricultural Sciences has conducted a long-term evaluation of the genetic factors breed and aptitude for milk yield, and TMR energy density on milk production, BW, BCS (Nielsen et al., 2003), body fat, and lipolytic responses (Theilgaard et al., 2002). Results from this evaluation have demonstrated that genetic selection has resulted in stronger lipolytic responses to energy deficiency in Danish Reds and Holsteins than in Jerseys. Feeding a TMR with higher energy density increased BCS, and breed × diet interactions exist for milk production. The present study compares Holstein-Friesian heifers with high or low genetic merit for milk, fat, and protein production under 4 different conditions: combinations of milking frequency (2 or 3 times a day) and feed caloric density (high or low). The experimental factors were selected for their strong effects on milk production, thus causing substantial differences in milk production levels, and because they create environments that model different management strategies in the range from extensive to intensive. The experimental design allows discrimination between the roles of genotype, environment, and

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Table 1. The compositions of the 2 rations fed to cows¹

	High caloric density ration (E)	Low caloric density ration (S)
Partial mixed ration (PMR) components (% DM of total PMR)	Corn silage (49%) Grass silage (30%) Soybeans, extracted/ customized meals (21%)	Grass silage (86%) Concentrates (14%)
PMR supply	Ad libitum	Ad libitum
PMR DM, g/kg of product	377	451
PMR NE _L , MJ/kg of DM	6.5	5.9
PMR DVE, ² g/kg of DM	77.5	65.0
PMR OEB, ² g/kg of DM	7.4	7.3
Concentrate supply, kg/d	8	3
Concentrate DM, g/kg of product	877	881
Concentrate NE _L , MJ/kg of DM	7.5	7.4
Concentrate DVE, g/kg of DM	119	112
Concentrate OEB, g/kg of DM	27	47

¹The high caloric density ration was balanced and attuned to expected mean milk production; the low caloric density ration was adapted to reduce energy content with minimal effects on other aspects of the ration.

²DVE = True protein digested in the small intestine; OEB = degraded protein balance.

G×E interactions in relationships between milk production level and energy and protein balances. Here, the effects of G×E interactions on milk yield, energy balance, and protein balance are assessed and the results help to determine if potential health risks in dairy cows are especially linked to high yield per se, genetic merit for high yield, management, or a mismatch between the latter 2 factors.

MATERIALS AND METHODS

Experimental Design

Treatments were applied in a 2 × 2 × 2 factorial arrangement. Heifers with a high genetic merit for fat and protein production (**H**) or a low genetic merit (**L**) were milked 2 times per day (**2**) or 3 times (**3**) and fed a relatively high caloric density ration (**E**, as derived from energy) or low caloric density ration (**S**, as derived from silage; Table 1). Prepartum and during the first 100 DIM, measurements were done to assess milk yield and energy and protein balances in H and L under different keeping conditions. Per combination of milking frequency and ration, 14 H cows were compared with 11 L cows. The slight imbalance was caused by difficulties in obtaining L heifers on farms that met our health requirements. Experimental groups were designated by frequency of milking (2 or 3), energy density of ration (E or S), and genetic merit group (H or L) to give 8 treatment designations: **2EH**, **2EL**, **2SH**, **2SL**, **3EH**, **3EL**, **3SH**, and **3SL**.

Animals and Keeping Conditions

The heifers were purchased from 61 farms across the Netherlands. Measures were taken to reduce the risk

of introducing novel pathogens into the research farm's herd. Farms from which the heifers were purchased had demonstrably low risks for harboring paratuberculosis, leptospirosis, or infectious bovine rhinotracheitis, as evidenced by certificates from specific disease eradication and prevention programs. Blood samples were collected from candidate experimental cows and screened for possible infections of infectious bovine rhinotracheitis, bovine virus diarrhea, leptospirosis, and *Salmonella*. Heifers that showed signs of disease, including skin lesions and leg or claw disorders, were barred from the research farm. One hundred heifers were used in this study. They arrived at the research farm on average 10.6 ± 0.8 (SEM) wk before calving. The latter occurred at a mean age of 2.2 ± 0.02 (SEM) yr from May 2003 through August 2004.

The heifers were sired by 53 different bulls and the size of paternal half-sibling groups was controlled to 6 individuals or less. The bloodlines of the heifers was 100% North American Holstein or mixed breed with 87.5% North American Holstein and 12.5% European Friesian-Holstein (n = 8). Cows were selected on the basis of the Dutch "Inet" EBV. Inet is the Dutch production index for milk, fat, and protein and is calculated as: 0.06 × EBV kg of milk + 0.7 × EBV kg of fat + 4.2 × EBV kg of protein; and is expressed in euros (€). The H cows had an average Inet value of 171, ranging from 112 to 241, and L cows had a mean Inet value of -24, ranging from -117 to 34. In a similar way, the mean EBV for kilograms of milk production were 771 (ranging from -6 to 1,704) and -68 (from -729 to 561) for H and L cows, respectively. The mean reliability of the EBV of the heifers' sires was 95%.

The heifers were housed indoors in 4 adjacent sections of a free-stall barn at research farm Nij Bosma

Zathe, the Netherlands. Each of the 4 sections, representing a combination of milking frequency and ration, consisted of 16 cubicles and 16 feeding troughs and housed both H and L heifers. Nonexperimental cows were added to each group up to a group total of 16 cows. The cows in the 2-times-daily groups were milked at 0600 and 1700 h, whereas those in the 3-times-daily groups were milked at 0600, 1400, and 2200 h. The free-stall floor was slatted concrete and cleaned by a manure scraper. Cubicles were equipped with rubber mats and a thin layer of sawdust. Water and partially mixed ration (**PMR**) were available ad libitum. The PMR was provided via a roughage intake control system, which allowed the monitoring of the daily roughage intake per individual. The roughage intake control system (Insentec, Marknesse, the Netherlands) comprises a feeding gate (which recognizes individual cows by their neck collars), and weighing troughs, which register the amount of PMR that cows eat during a meal. Concentrates were provided individually via feeding boxes and in the milking parlor. Cows in the E groups had access to a high caloric density PMR and 8 kg of concentrate per day (Table 1). Those in the S groups had access to a low caloric density PMR and received 3 kg of concentrate per day. The composition of the ration for the E groups, which was well balanced and attuned to expected mean milk production, was adapted for the cows in the S groups in such a way that energy content would be reduced with minimal effects on other aspects of the ration. Obviously, the 2 rations differed for multiple characteristics (see Table 1), but here these differences are referred to as variation in "caloric density."

Measurements

On an individual level, milk production was recorded every milking. Fat, protein, and lactose contents in milk were measured weekly in composite milk samplings from successive milkings over 2 d. Weighing platforms were used for automatically monitoring BW daily. Body condition was scored every 2 wk starting in lactation wk 1 by a single experienced farm assistant. Intake of roughage and concentrates was automatically registered per individual cow and used to calculate daily intakes. Additionally, a set of parameters was measured to assess blood metabolite and hormone profiles, locomotory function, udder health, and immune function, but these will be presented elsewhere.

Calculations

Milk production was expressed as ECM: $(0.337 + 0.116 \times \text{fat content} + 0.06 \times \text{protein content}) \times \text{kg of}$

milk. Energy balance (**EB**) was expressed as NE_L in megajoules and calculated as: $DMI \text{ per day} \times NE_L \text{ in total ration} - \{[(42.4 \times BW^{0.75} + 442 \times ECM) \times (1 + (ECM - 15) \times 0.00165)] + 660\} \times 0.0069$, where +660 is the correction for growth in heifers and the factor $\times 0.0069$ converts the Dutch energy unit VEM into megajoules. For information on the Dutch energy (VEM) system, see Van Es (1978). Protein balance (**PB**, in g/d) was calculated as intake of true protein digested in the small intestine (**DVE**) minus requirement DVE: $\{54 + [0.1 \times BW]\} + \{1.396 \times [(milk \text{ production in kg/d}) \times (milk \text{ protein in } \%) \times 10]\} + \{0.000195 \times [(milk \text{ production in kg/d}) \times (milk \text{ protein in } \%) \times 10]^2\}$. For information on the Dutch protein (DVE/OEB) system, see Tamminga et al. (1994). Postpartum empty body weight (**EBW**) was expressed as $BW - (DMI \times 4)$ to correct for gut fill (Jarrige, 1989).

Statistical Analyses

The data were analyzed with REML-type analyses by use of ASREML (Gilmour et al., 2004). Fixed effects in the model were milking frequency (2 or 3), caloric density of the ration (S or E), genetic merit (L or H), and interactions among these, week of lactation (1 to 14), and the time since the start of the experiment (wk 1 to 81). The latter functioned to minimize bias originating from time-related variations (e.g., seasonal fluctuations) and will not be discussed further. Time effects were fitted as splines and effects of lactation week were fitted per combination of milking frequency, caloric density of the ration and genetic merit. Thus, the model for statistical analyses was:

$$Y_{ijklmn} = \mu + MF_i + QR_j + GM_k + in_{ijk} + f(WS_l) + f(WL_{ijkm}) + AN_n + E_{ijklmn}$$

where Y_{ijklmn} is the variable for milking frequency i , caloric density j , genetic merit k in lactation week m , in week l since the start of the experiment and animal n ; MF_i is the milking frequency (2 or 3 times a day); QR_j is the caloric density of the feed ration (relatively high or low); GM_k is the genetic merit (high or low genetic merit for fat and protein production); in_{ijk} represents all interactions between the 3 fixed effects; $f(WS_l)$ is a spline describing the effect of the weeks passed since the start of the experiment; $f(WL_{ijkm})$ is a spline describing the effect of the weeks passed since the start of the lactation; and AN_n is the individual animal (included as a random effect).

Results presented here are the means ($\pm$ SEM) predicted by the described statistical model. In the results section, effects and differences between groups are re-

Figure 2. Mean values ($n = 100$) of percentages of protein (●, left y-axis), fat (□, right y-axis), and lactose (△, right y-axis) in milk during the first 14 wk of lactation. Changes in time were significant for protein and fat.

Ratios decreased gradually from 1.46 ± 0.02 in wk 1 to 1.38 ± 0.02 in wk 9 and thereafter stabilized around this value. Lactose content was higher ($P < 0.001$) in the milk of cows in E groups ($4.78 \pm 0.02\%$) than in S groups (4.63 ± 0.02). Fluctuations in milk content during lactation occurred for fat and protein (P -values < 0.001) and are illustrated in Figure 2.

DMI

Dry matter intake was higher ($P < 0.001$) in cows in E groups (20.3 ± 0.3 kg/d) than in S groups (16.1 ± 0.3) and, during the first weeks of lactation, increased ($P < 0.001$) over time (Figure 3). Only within the groups that were milked 2 times daily did the H cows (18.7 ± 0.3 kg/d) have a higher (P -value for 2-way interaction < 0.05) DMI than the L cows (17.7 ± 0.4).

Energy and Protein Balance

Calculated EB were lower ($P < 0.001$) in cows that were milked 3 times (-13.3 ± 1.7 MJ/d) than in those that were milked 2 times (-5.3 ± 1.7 MJ/d). Cows in E groups had a mean EB of -6.0 ± 1.7 MJ/d, which was higher ($P < 0.001$) than the -12.6 ± 1.7 MJ/d in cows in S groups. In the E groups, but not the S groups, H cows tended (interaction genetic merit $\times$ ration: $P = 0.07$) to have a lower EB (-6.5 MJ/d) than the L cows. Like calculated EB, calculated PB were lower ($P < 0.001$) in cows that were milked 3 times (-85.4 ± 23.4 g/d) than in those that were milked 2 times (10.9 ± 23.5

Figure 3. Mean ($n = 50$) DMI during the first 14 wk of lactation for heifers that were fed low caloric density rations (●) or high caloric density rations (□). Differences between the groups and changes in time were significant.

g/d). Cows in E groups had higher ($P < 0.01$) PB (-7.8 ± 23.4 g/d) than cows in S groups (-66.6 ± 23.6 g/d). The effect of genetic merit ($P < 0.01$) on PB merely reflected that PB in the EL groups were on average 142 g/d higher (P -value for 2-way interaction < 0.01) than in the EH, SL, and SH groups. High caloric density rations improved PB, but only in L cows. Alternatively, the latter had a better PB than H cows, but only when a high caloric density ration was fed. Changes in calculated EB and PB over time (P -values < 0.001) are illustrated in Figure 4.

BCS

The mean postpartum BCS was 2.78 ± 0.04 ; with a lower ($P < 0.01$) mean score for H cows (2.71 ± 0.04) than for L cows (2.85 ± 0.05). The mean BCS prepartum, assessed on average 1.6 wk before calving, was 3.58 ± 0.03 , with the same predicted scores for H and L cows. Postpartum EBW was on average 445.3 ± 4.2 kg and affected only by week of lactation ($P < 0.001$). Mean weights decreased from 476.8 ± 4.6 kg in wk 1 to 444.7 ± 4.3 in wk 7, after which weights increased gradually to 449.7 ± 4.5 kg in wk 14. Uncorrected postpartum BW was on average 518.1 ± 4.5 kg. Unlike EBW, uncorrected postpartum BW was not affected by week of lactation. Interactions between milking frequency and genetic merit, which were near significance for EBW ($P =$

Figure 4. Mean values ($n = 100$) for calculated energy balances (EB, ●, left y-axis) and calculated protein balances (PB, □, right y-axis) during the first 14 wk of lactation. Changes in time were significant for both EB and PB.

0.07), affected BW ($P < 0.05$), with cows in 3L groups being heavier than those in 3H groups. Mean postpartum BW were on average 535.3 ± 8.5 and 508.3 ± 7.5 kg, respectively, compared with 511.7 ± 8.5 and 516.9 ± 7.7 kg for cows in the 2L and 2H groups.

DISCUSSION

Dairy cow health affects animal welfare, animal productivity, zoonotic risks, and international trade. This study investigated whether selection for milk yield affects the risk for negative energy and protein balances, which may be associated with health disorders. Heifers with high or low genetic merit for milk yield were exposed to experimental factors that, a priori, were assumed to create substantial differences in milk production levels and model different management strategies in the range from extensive to intensive. This allowed the assessment of the effects of genotype environment and G×E interactions in relationships between milk yield and animal health. The experimental conditions were successful in creating substantial contrasts among groups in terms of genotypes, environments and, subsequently, milk production levels. The difference in Inet EBV between the high and low genetic merit cows was €195, on average. With an average genetic trend of about +€20/yr in the Netherlands, this represents a period of 10 yr of ongoing selection. The 2 rations differed considerably in energy content although they were within the range of normal rations used on Dutch farms, and caused a significant difference in DMI (4.2

kg/d). The differences in rations caused substantial differences in EB, PB, and milk production levels (9.6 kg of ECM/d). Milking 3 times daily instead of 2 times increased yield (4.1 kg of ECM/d), although like the stimulating effects of high genetic merit (3.5 kg of ECM/d), this required the provision of high caloric density feed. The experimental factors caused mean daily ECM productions in the first 100 DIM to vary between 21.8 and 35.2 kg, which represents a substantial contrast as mean daily ECM production levels of Dutch Black and White dairy cows (across 305 d) were 19.5 and 28.8 kg in 1985 and 2004, respectively. The experimental factors affected milk production in the presumed direction. Milk productions were lowest in the L cows that were fed the S ration and milked 2 times daily and highest in the H cows that were fed the E ration and milked 3 times a day. The discriminative power of the study may be illustrated by the minimal difference that lead to significance for both main effects and G×E interactions. Power calculations ($\alpha = 0.05$, $\beta = 0.10$) resulted in minimal differences of about 10 and 20% of the mean ECM, respectively, with similar findings for EB. For ECM this means that contrasts between levels of main factors of about 3 kg/d or more were significant. For differences between combinations of genotype, milking frequency, and feed caloric density, this was about 6 kg/d or more and this relatively low power may have attributed to the absence of significant 3-way interactions.

Effects of Genotype

Significant effects of genetic merit for milk yield, independent of other factors, occurred for postpartum BCS with significantly lower scores in H cows than in L cows. A positive genetic correlation exists between milk production level and loss of body condition in early lactation (Berry et al., 2002). Changes in BCS reflect the energy status of cows and several studies associated high genetic merit with strong NEB (Veerkamp et al., 1995). Increased growth hormone concentrations in high genetic merit cows are likely to play a role (Veerkamp et al., 2003), for example, as growth hormone inhibits insulin-induced lipogenesis and thus influences the partitioning of resources in the body. Strikingly, the lower BCS in H cows than L cows was not accompanied by a stronger calculated NEB in H cows. Inaccuracies in calculating EB will have played a role (see next section), although a biological cause cannot be excluded. The H cows may have relatively high energy expenditures for maintenance and differ from L cows in the way food resources are utilized and allocated to body functions.

Effects of Environment

Ration. Ration was the most determining factor affecting milk production, with decreased milk production when the caloric density was low (-9.6 kg of ECM/d). In part, this decrease in ECM was caused by significantly reduced protein content (-0.26%). The low caloric density ration significantly reduced milk lactose (-0.15%), but not fat content, resulting in an increased fat to protein ratio ($+0.11$). The results are not surprising as the provision of less concentrate typically decreases DMI, increases fat content, and decreases protein content (Brocard and Bareille, 2004). Higher energy intakes from supplementation with cereal grains, as for the cows in the E groups in this study, promotes ruminal propionate and microbial protein more than acetate and this increases the mammary synthesis of lactose and protein to a higher degree than fat (Walker et al., 2004). In addition to the energy supply, the direct protein supply will have affected protein content. Dry matter intake and calculated PB were significantly higher in cows in E groups than in S groups ($+58.8$ g/d), and increased intake of CP, at least relative to conditions of low protein intake, will increase protein content (Walker et al., 2004). The temporal patterns in milk production and content as shown in Figures 1 and 2 are in line with earlier studies reviewed by Walker et al. (2004). Because of dilution, fat and protein concentrations tend to be lowest around 40 to 60 d postpartum when lactose and milk productions are relatively high.

In line with the fat to protein ratios and calculated PB, the calculated EB were significantly lower in cows in the S groups than E groups (-6.6 MJ/d). The impact of the feed treatment overruled the typical inverse relationship between milk yield and energy balance within a more or less homogeneous setting (Veerkamp et al., 1995). Others have reported that EB was more strongly controlled by feed intake than by milk production (Beam and Butler, 1999); this could apply especially to early lactation when cows have difficulty matching feed intake to energy expenditure. Calculated EB is typically most negative within the first 12 d postpartum and increases above zero after approximately 70 d postpartum (De Vries et al., 1999). The latter corresponds with EB becoming positive in lactation wk 11 in our cows in the E groups. Cows in the S groups typically remained in NEB throughout the 100-d experimental period and this marks these animals as having strong NEB compared with the normal situation.

The mean cumulative balances for energy and protein were -930 MJ/100 d and $3,720$ g/100 d, respectively, and during the first 100 DIM the heifers lost, on average, 27.1 kg of EBW (as derived from Table 2). This amounts to energy and protein values per kilogram of

mobilized body reserves of 34.3 MJ and 137 g, respectively. Such values are within the wide range of expected values (Tamminga et al., 1997), although the calculated energy deficit is high relative to loss of EBW. Compare, for example, the 34.3 MJ/kg of mobilized body reserves (present study) with the 30.9 MJ/kg during the first 8 wk of lactation as reported by Tamminga et al. (1997). One unit of fat from body tissue contains more energy than one unit of protein from body tissue and the relatively little loss of EBW compared with calculated NEB indicates that the heifers mainly mobilized fat. The variation in the aforementioned values will in part result from inaccuracies. The systems used for calculating energy balances, like the Dutch VEM system (Van Es, 1978), are based on averages found for cows used in given feeding trials. Variation in feed composition and individual traits of cows, like their rumen flora and papillae, will cause inaccuracies in the estimation of the cows' energy balance (Bruinenberg et al., 2002). Also, cows differ with regard to the partitioning of energy between different physiological systems; cows with similar energy intakes and expenditures via the milk may actually experience a different EB.

Milking Frequency. Milking frequency affects the rate of cell death by apoptosis, like the degree of oxidative stress imposed by feed and the tissue's ability to handle damage induced by reactive oxygen species (Stefanon et al., 2002). Milking more frequently may shift the balance between cell proliferation and cell removal in a way that increases the number of mammary cells and favors milk yield. For example, by local enhancement of prolactin receptors and by increased prolactin-mediated inhibition of local IGF binding protein-5 production, which would otherwise abolish the cell-saving actions of growth hormone-induced IGF-I (Flint et al., 2001). Thus, increased milking frequency promotes milk yield and persistency of lactation; for example, by counteracting the negative effects of oxidative stress. Here, we found that increased milking frequency increased milk yield, although only significantly when the cows received high caloric density rations. This indicates that in the S groups, nutrient intake was limiting for milk production. Milking the cows 3 times a day instead of 2 times significantly decreased EB. More-frequent milking lowered protein content (-0.18%), fat content (-0.23%), calculated EB (-8 MJ/d), and calculated PB (-96.3 g/d). Environmental factors like milking frequency and feeding management, more than milk production level per se, strongly predicted the cows' energy balances.

Effects of G×E Interactions

Different genotypes are not always equally affected by different environments (Falconer, 1952), and across

different environments such G×E interactions can result in different magnitudes of variance (scaling) as well as differences in ranking of the genotypes (reranking). Genotype × environment interactions in dairy cattle have been demonstrated at Langhill Dairy Cattle Research Centre in Scotland (Veerkamp et al., 1994) and Moorepark Research Centre in Ireland (Dillon et al., 2006). Especially under conditions that promote high-yield, high genetic merit cows have increased tissue mobilization, DMI, and, as a result, milk production (Veerkamp et al., 1995). This corresponds with our findings. High genetic merit compared with low genetic merit (mean difference of 3.5 kg of ECM/d), as well as 3 times milking compared with 2 times milking (mean difference of 4.1 kg of ECM/d), significantly increased milk production, but only in the groups that received high caloric density rations. The G×E interactions indicate that in the cows that received low caloric density rations, the low intake of resources limited the effects of factors that normally promote milk production. Similarly, Hoogendoorn et al. (1990) found that grazed cows showed milk responses to growth hormone only when the quality of the grass was relatively good. Earlier findings predict that our H cows should have had a stronger NEB than L cows, at least in the E groups (Veerkamp et al., 1995). This effect (6.5 MJ/d) proved to be near significance ($P = 0.07$). The expected higher DMI in H cows compared with L cows under yield-promoting conditions (i.e., in the E groups) was not found. The H cows did have higher DMI than the L cows, but only in the groups that were milked 2 times daily. This influence of milking frequency is difficult to explain. Interactions between milking frequency and genetic merit were detected also for postpartum BW, with L cows being heavier than H cows in the groups that were milked 3 times daily. This was near significance for EBW and seems to correspond with the relatively low BCS of H cows. The higher fat content in H cows than L cows, but only in the S groups, mirrors relatively high fat output in the H cows under severe conditions. Differences in fat content between cows in the HS and LS groups existed from lactation wk 1 and increased somewhat over time, indicating a tendency of high genetic merit animals to mobilize fat reserves for fat production, although diet-related variation in ruminal acetate production may have played a role. The relatively favorable calculated PB for cows in the EL groups means that under favorable conditions of sufficient nutrient intake, the balance between allocating protein to maintenance or milk production tips more toward the former in low than in high genetic merit cows. High genetic merit cows seem more prone to allocate resources toward high fat output in times of nutrient-deficient diets and toward high protein output with

high nutrient diets. The relatively low milk productions by cows in the 2L groups compared with the 2H, 3L, and 3H groups indicates that a first yield-promoting measure, be it high genetic merit or an extra milking, renders the most marginal benefit compared with additional ones.

High Yield and Animal Health

High genetic merit for milk yield has been associated with poor energy balance, increased disease susceptibility (Pryce et al., 1998), and decreased fertility (Veerkamp et al., 2003), although not in all studies (Knight et al., 2004). The high genetic merit cows in this study were more prone to allocate resources toward milk production than low genetic cows and had lowered postpartum BCS. Low BCS and high dairy form (angularity) are genetically correlated with reduced cow health (Dechow et al., 2004) and strong decreases in postpartum BCS decrease fertility (Gillund et al., 2001). High genetic merit for milk yield seems intrinsically connected to increased risks for disorders related to severe NEB, although in this study management factors such as feed caloric density and milking frequency had the stronger impact on calculated EB and PB. Low nutrient input reduced energy balances, as did an extra milking, but high nutrient input may have negative side effects as well. Sehested et al. (2003) compared groups of cows fed a diet of clover grass supplemented with different amounts of concentrates (0, 19, or 38% of DMI), and the lowest incidences of health disorders were associated with the group that did not receive concentrates. Mismanagement can be a more potent risk factor for animal health than genetic ability for milk yield, although one-sided selection for high milk yield is likely to render cows that are increasingly more vulnerable to disease and poor fertility (Olteneacu and Algers, 2005). Keeping such animals by farmers that have increasingly less time available per cow because of scaling-up of farms, at least in the Netherlands, demands increasingly more management skills. In recent years, the tendency for one-sided selection on high yield has been weakened (Miglior et al., 2005) and this change is supported by the present findings.

Clear G×E interactions existed for milk production level, which increases the likelihood that such interactions play a role in the relationship between milk yield and animal health. Others reported on such interactions. Windig et al. (2005) found that cows in high-producing herds had better udder health than those in low-producing herds, but within the former, the highest producing cows had the poorest udder health. In situations of inferior management, unfavorable genetic correlations between milk yield and somatic cells scores

or conception rate are relatively strong. From different studies, Oltenacu and Algers (2005) concluded that negative genetic correlations between production and fitness traits increase in less favorable environments and that selection for increased yield has decreased the adaptability of modern dairy cows. The present findings indicate a relatively strong tendency of high genetic merit cows to allocate resources toward milk production at the cost of maintenance, but there was little evidence that these animals are especially at risk for severe negative EB and PB during low nutrient supply. High genetic merit cows may have been selected to reach a certain limit in terms of BCS loss and NEB, but like low genetic merit cows, reduce yield in response to low nutrient supply beyond that point. The assumption that high genetic merit cows especially have difficulty in coping with extensive keeping conditions is not confirmed by the results presented here because, for example, they lower milk production levels in response to low nutrient intake in the same way as low genetic merit cows.

Signs for increased health risks, like severe negative EB and PB or low BCS, were not concentrated in the highest producing cows that were in the 3EH group or E groups. The production levels were apparently within the cows' metabolic capacities, which more than their mammary glands' capacities limits milk yield. High milk production levels per se need not compromise the health status of cows, although a negative correlation with EB exists and this is linked to minor changes in the hypothalamic-pituitary axis (Beerda et al., 2004). The latter indicates that high yield does represent allostatic load and triggers allostatic responses, but does not compromise the functioning of biological systems such as the immune system. In older cows, the situation may be different because of accumulated load.

CONCLUSIONS

The results support the view that high genetic merit for milk yield is intrinsically connected to increased risks for NEB-related disorders, although feed caloric density and milking frequency had the stronger impact on energy and protein balances, which underlines the impact that mismanagement can have on animal health risks. Ongoing one-sided selection for high yield can be combined with good animal health, but at the cost of increasing demands on farmers' time and management skills. Genotype $\times$ environment interactions played a role in the present study, but specific health parameters need to be evaluated to determine if cows of different genetic merit for milk yield are differently at risk for disease under intensive and extensive management conditions. Present and earlier findings indicate that

high milk production levels per se need not compromise the health status of cows. High yield will increase allostatic load, however, and it cannot be excluded that accumulated physiological load across multiple lactations compromises disease resistance in older cows. Prospective publications will deal with the specific health indicators that were measured during this study and link these to the basic findings reported here.

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