Analysis of the genetic architecture of energy balance and its major determinants dry matter intake and energy-corrected milk yield in primiparous Holstein cows

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ABSTRACT

The focus of modern dairy cow breeding programs has shifted from being mainly yield based toward balanced goals that increasingly consider functional traits such as fertility, metabolic stability, and longevity. To improve these traits, a less pronounced energy deficit postpartum is considered a key challenge. On the other hand, feed efficiency and methane emissions are gaining importance, possibly leading to conflicts in the design of breeding goals. Dry matter intake (DMI) is one of the major determinants of energy balance (EB), and recently some efforts were undertaken to include DMI in genomic breeding programs. However, there is not yet a consensus on how this should be achieved as there are different goals in the course of lactation (i.e., reducing energy deficit postpartum vs. subsequently improving feed efficiency). Thus, the aim of this study was to gain more insight into the genetic architecture of energy metabolism across lactation by genetically dissecting EB and its major determinants DMI and energy-corrected milk (ECM) yield at different lactation stages applying random regression methodology and univariate and multivariate genomic analyses to data from 1,174 primiparous Holstein cows. Daily heritability estimates ranged from 0.29 to 0.49, 0.26 to 0.37, and 0.58 to 0.68 for EB, DMI, and ECM, respectively, across the first 180 d in milk (DIM). Genetic correlations between ECM and DMI were positive, ranging from 0.09 (DIM 11) to 0.36 (DIM 180). However, ECM and EB were negatively correlated ($r_g = -0.26$ to -0.59). The strongest relationship was found at the onset of lactation, indicating that selection for increased milk yield at this stage will result in a more severe energy deficit postpartum. The results also indicate that EB is more affected by DMI ($r_g = 0.71$ to 0.81) than by its other major

determinant, ECM. Thus, breeding for a higher DMI in early lactation seems to be a promising strategy to improve the energy status of dairy cows. We found evidence that genetic regulation of energy homeostasis is complex, with trait- and lactation stage-specific quantitative trait loci suggesting that the trajectories of the analyzed traits can be optimized as mentioned above. Especially from the multivariate genomic analyses, we were able to draw some conclusions on the mechanisms involved and identified the genes encoding fumarate hydratase and adiponectin as highly promising candidates for EB, which will be further analyzed.

Key words: energy balance, dry matter intake, energy-corrected milk yield, genetics, genomics

INTRODUCTION

In the recent past, dairy cattle breeding programs have broadened and adapted to cover functional traits. However, selection is still mainly for high milk yield, resulting in a partial shift of available energy toward milk production at the cost of other energy-consuming processes, especially those that are scarcely considered in breeding goals (e.g., reaction to pathogens; Rauw et al., 1998). This is worsened by the fact that energy expenditure is higher than energy intake in early lactation (Bauman and Currie, 1980). Therefore, exclusive breeding for high milk yield will intensify the energy deficit postpartum. This, in turn, leads to undesirable side effects for immunological and reproduction traits and consequently for animal welfare (Rauw et al., 1998).

Genomic selection has the power to increase production levels even faster than in the past, which may result in more dramatic consequences for functional traits that are not under selection. For the dairy research herd Karkendamm, the severity and duration of the energy deficit postpartum has increased remarkably over a relatively short period of time (von Leesen et al., 2014), and we hypothesize that this is at least partly due to the increased intensity and accuracy of selection for milk production. However, genomic information can

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also be used to decrease the energy deficit in early lactation, thereby improving health and fertility.

Genomic selection for DMI, which is the major determinant of energy balance (**EB**; Hüttmann et al., 2009), is challenging because the trait is difficult and costly to measure; therefore, the reference population is small even though international collaboration has been established to increase its size. However, using the data set of the global Dry Matter Initiative, which represents the largest existing international database consisting of individual dairy animal DMI records from Europe, North America, and Australasia (Berry et al., 2014) and their associated genotypes (Pryce et al., 2014), de Haas et al. (2015) demonstrated that genomic prediction of DMI is possible with a mean prediction accuracy of 0.44. Although accuracy of genomic selection for DMI is less good than that for production traits, this can be considered as great progress because we were unable to include this trait in breeding goals until we entered the genomics era.

In the study of de Haas et al. (2015), predicted DMI at DIM 70 was used as the phenotype because this was the point of time when the largest number of actual DMI observations existed within the complete data set (see also Berry et al., 2014). For future data collection, we need to determine the optimal phenotype and a strategy for data recording. Continuous recording of DMI throughout the whole lactation is expensive and not absolutely necessary; however, DMI should be measured at more than one lactation stage for 2 reasons: (1) Tetens et al. (2014) found out that genetic regulation of DMI differs between early and mid lactation, and (2) selection for DMI has to be seen in the context of conflicting requirements regarding short-term efficiency, which can be improved by an appropriate reallocation of energy, and long-term efficiency, which requires changes of acquisition (Puillet et al., 2016). In early lactation, DMI should be increased to breed for well-conditioned animals with less severe energy deficits and, consequently, fewer physiological and immunological problems, which are a limiting factor for longevity and the profitability of milk production. During mid and late lactation, cows should consume less feed or, in other words, a reasonable amount of feed that matches their milk production. Feed represents a major proportion of the variable costs in dairy production; therefore, an optimization of the overall feed efficiency is crucial with regard to economic efficiency.

For the inclusion of DMI in breeding goals, we need better insights into the genetic architecture of EB, DMI, and milk yield. On the one hand, exact knowledge on heritability estimates and genetic correlations in different lactation stages might be the key to a successful redefinition of breeding programs. On the other

hand, knowledge of genes influencing 1, 2, or all 3 of these traits simultaneously may provide deeper insights into the underlying regulatory circuits. The aim of the current study was therefore to examine the degree and change in heritability of EB and its major determinants (DMI and ECM yield) across lactation and to estimate genetic correlations among EB, DMI, and ECM using random regression. Furthermore, deregressed EBV for all 3 traits were estimated and the results for DIM 11 and for every fifth lactation day ranging from DIM 15 to 180 were used as phenotypes for univariate and multivariate genome-wide association studies (**GWAS**) with the aim of identifying genomic regions with a significant effect on EB, DMI, or ECM at specific or multiple stages of lactation. By using this approach to investigate genetic relationships between the aforementioned traits, it is possible to identify possible pleiotropic effects on different energy-related traits, deduce mechanistic hypotheses and candidate genes, and determine whether remodeling of the lactation curve trajectory of DMI is a possible strategy to improve both energy status and production efficiency.

MATERIALS AND METHODS

Animals and Phenotypes

Data from 1,174 primiparous Holstein Friesian cows descending from 286 sires and including 191 daughter–dam pairs were recorded between September 2005 and December 2012 on the dairy research farm Karkendamm (Institute of Animal Breeding and Husbandry, Christian-Albrechts-University, Kiel, Germany). This farm ran a bull dam performance test when data were recorded, and all bull dam candidates had to complete a test period under commercial conditions in a freestall barn until DIM 180. Nonqualified primiparous cows left the herd afterward. Thus, only records between DIM 11 (start of feed intake recording) and DIM 180 were used in the present investigation.

Cows were milked twice per day, and milk yield was automatically recorded at every milking. Milk composition was analyzed weekly based on samples collected from 2 consecutive milkings. Energy-corrected milk was calculated according to Kirchgeßner (1997):

$$\text{ECM (kg)} = \text{milk yield (kg)} \times \frac{(0.39 \times \text{fat \%} + 0.24 \times \text{protein \%} + 0.17 \times \text{lactose \%})}{3.17}$$

For this purpose, milk composition per day was obtained by weighting the respective or previous analysis values per day with the respective milk yield.

Figure 1. Data distribution across DIM 11 to 180 for ECM, BW, DMI, and energy balance (EB).

Cows were weighed after every milking since March 2006. Morning and evening BW were averaged to derive a daily value.

Animals were fed a TMR diet ad libitum. Daily TMR intake was recorded for each animal via single feeding troughs equipped with a weighing unit and automatic cow identification. Because cows were generally housed separately during the first 10 DIM, no feed intake data were available for this period. Individual TMR intake was deleted for the first and last day with TMR intake information and when cows were separated for insemination or pregnancy testing. Values obtained for fresh TMR intake were discarded if they were among the 0.5 percentage highest or 0.5 percentage lowest values, resulting in a range of fresh TMR intake between 4.8 and 77.3 kg. Dry matter content of the TMR was analyzed twice weekly. The average lactation net energy of TMR was 7.1 MJ/kg of DM. Fixed amounts of concentrates were dispensed via concentrate feeders. Energy balance was calculated as the difference between energy intake and energy requirements for milk production and maintenance based on feed intake, milk yield and constituents, and the daily weights as described by Buttchereit et al. (2010).

Records for a specific trait were excluded from the analyses if the number of observations per cow for this trait across the entire period was less than 4. This resulted in 824 primiparous cows with EB data, 910 cows with DMI records, and 1,174 cows with ECM records. Figure 1 shows the data distribution across DIM 11 to 180 for ECM, BW, DMI, and EB. The number of ob-

servations per DIM was lowest in early lactation for all analyzed traits. From the third week of lactation (ECM and BW) and fourth week of lactation (DMI and EB) on, data were relatively evenly distributed. A summary of the data is given in Table 1. In addition, Figure 2a to d shows the trend of the mean for ECM, BW, DMI, and EB across DIM 11 to 180 and the standard deviations for the traits throughout DIM 11 to 180. The average EB was negative until DIM 55 and stabilized between DIM 55 and 63 (switching from positive to negative and vice versa during this period). It should be noted, however, that the duration of the energy deficit varied largely across cows and that some cows did not change into a state of positive EB throughout the entire observation period (see also von Leesen et al., 2014).

Statistical Analysis

A pedigree file was created by tracing the pedigree of cows with data 3 generations back. The resulting file consisted of 5,683 animals. Data were analyzed using a random regression animal model considering the fixed effects of test day, age at first calving, and stage of lactation. Age at first calving was divided into 5 classes (21–25, 26, 27, 28, and 29–38 mo). The general lactation curve was modeled by the function according to Ali and Schaeffer (1987). The random regression coefficients for permanent and additive genetic effects were modeled applying Legendre polynomials of 2 degrees. For EB and DMI, second-degree polynomials worked best, and we decided to choose the same model for all

Table 1. Descriptive statistics for energy balance (EB), DMI, ECM, fat content, protein content, lactose content, and BW (first lactation, DIM 11–180)

Trait	n	Mean	SD	Minimum	Maximum
EB (MJ of NE _L /d)	68,436	5.16	35.86	−141.80	185.59
DMI (kg/d)	93,797	20.17	4.83	1.99	45.45
ECM (kg/d)	165,805	32.72	4.69	2.50	53.64
Fat (%)	24,138	3.64	0.59	1.22	8.68
Protein (%)	24,138	3.24	0.24	1.96	6.01
Lactose (%)	24,237	4.88	0.15	3.17	5.38
BW (kg)	130,452	600.03	54.48	404.00	847.00

3 traits. Using higher orders would have led to convergence problems. The resulting model was

$$y_{ijklm} = \text{TD}_i + \text{AFC}_j + \sum_{n=1}^4 b_n x_{ijklmn}(d) + \sum_{n=0}^2 p_{kn} z_{ijklmn}(d) + \sum_{n=0}^2 a_{ln} z_{ijklmn}(d) + e_{ijklm},$$

where y_{ijklm} = the m th observation of EB, DMI, or ECM; TD_i = fixed effect of test day i ($i = 1$ –1,714 for EB, $i = 1$ –2,023 for DMI, and $i = 1$ –2,649 for ECM); AFC_j = fixed effect of age at first calving ($j = 1$ –5); b_n = fixed regression coefficients on lactation day d , with $x_{ijklm1}(d) = d/190$, $x_{ijklm2}(d) = (d/190)^2$, $x_{ijklm3}(d) = \ln(190/d)$, and $x_{ijklm4}(d) = [\ln(190/d)]^2$; p_{kn} = random regression coefficients for the permanent environmental effect of the k th animal with phenotypic records; a_{ln} = random regression coefficients for the additive genetic effect of animal l in the pedigree, with $z_{ijklm0}(d) = 1$, $z_{ijklm1}(d) = -1 + 2(d - 11/d_{\max} - 11)$, and $z_{ijklm2}(d) = 0.5\{3[-1 + 2(d - 11/d_{\max} - 11)]^2 - 1\}$ ($k = 1$ –824 for EB, $k = 1$ –910 for DMI, and $k = 1$ –1,174 for ECM; $l = 1$ –5,683); and e_{ijklm} = random error.

Residual errors were expected to have heterogeneous variances, so the first 180 DIM were split into 6 measurement error classes (11–30, 31–60, 61–90, 91–120, 121–150, and 151–180 DIM). Within these classes, residual errors were assumed to be homogeneous. Residual covariances were assumed to be zero.

Variance components were estimated univariately and bivariate by REML using the software package ASReml 3.0 (Gilmour et al., 2009). Approximate standard errors for daily heritability and daily repeatability estimates and genetic correlations across lactation were calculated with the method proposed by Fischer et al. (2004). ASReml 3.0 was also used for the estimation of EBV for EB, DMI, and ECM.

Genomic Analyses

A total of 876 animals with phenotypic information were genotyped using the Illumina SNP50v1 BeadChip

comprising a total of 54,001 SNP markers. The amount of missing genotypes per individual was <1% on average, and no animal had to be removed due to poor genotyping quality. Loci with >10% missing genotypes, a minor allele frequency <0.025, or unknown genomic position based on the bovine genome assembly UMD 3.1 (https://oct2018.archive.ensembl.org/Bos_taurus/Info/Annotation) were excluded, resulting in a final set of 40,583 informative markers. Quality control and data preparation were done with the plink package (Purcell et al., 2007).

Genome-wide association studies were carried out as described by Tetens et al. (2014) applying a linear mixed model approach using the freely available software tool GEMMA (genome-wide efficient mixed model association; Zhou and Stephens, 2012), with the daily EBV for DIM 11 and for every fifth day ranging from DIM 15 to 180 as phenotypes. These EBV were deregressed by dividing them by their respective reliability. For each marker, GEMMA fits the following mixed model:

$$\mathbf{y} = \mathbf{W}\boldsymbol{\alpha} + \mathbf{X}\boldsymbol{\beta} + \mathbf{u} + \boldsymbol{\varepsilon},$$

where $\mathbf{y}$ is an $n \times 1$ vector of deregressed EBV for n cows; $\mathbf{W}$ is an $n \times c$ incidence matrix of covariates, where c is the number of covariates; $\boldsymbol{\alpha}$ is a vector of corresponding coefficients including the intercept; $\mathbf{X}$ is an $n \times 1$ vector of marker genotypes at the locus tested and $\boldsymbol{\beta}$ is the corresponding effect size; and $\mathbf{u}$ is a vector of random genetic effects, with $\mathbf{u} \sim N(0, \mathbf{A}\sigma_g^2)$, where σ_g^2 represents the genetic variance and $\mathbf{A}$ is the relationship matrix. Here, the genomic relationship matrix was calculated with the freely available GCTA (genome-wide complex trait analysis) software tool according to Yang et al. (2011). Finally, $\boldsymbol{\varepsilon}$ is a random residual term, with $\boldsymbol{\varepsilon} \sim N(0, \mathbf{I}\sigma_\varepsilon^2)$, where σ_ε^2 represents the residual variance and $\mathbf{I}$ represents an identity matrix. The alternative hypothesis of H_1 ($\boldsymbol{\beta} \neq 0$) was tested against the null hypothesis H_0 ($\boldsymbol{\beta} = 0$) using the Wald test.

Besides the univariate analyses, bi- and multivariate analyses as also implemented in GEMMA (Zhou

and Stephens, 2014) were conducted using the same deregressed EBV as in the univariate analyses. Both uni- and bivariate GWAS models were also fitted without including the marker effect to be tested (termed null models later) to estimate the variance explained by all markers (chip heritability) and the genomic correlations between traits for the same DIM, respectively.

To account for multiple testing, the modified Bonferroni approach (simpleM) of Gao et al. (2008) was applied, which accounts for the fact that association tests in genome-wide studies are not independent due to linkage disequilibrium between markers. Based on a pairwise marker correlation matrix, the number of effective tests to be used in a subsequent Bonferroni correction is estimated, which is less computationally demanding compared with permutation procedures (Gao et al., 2010).

RESULTS AND DISCUSSION

Daily Repeatability and (Chip) Heritability Estimates for EB, DMI, and ECM

Figure 3 shows repeatability and heritability estimates for EB, DMI, and ECM across the first 180 DIM. The highest repeatability was found for ECM. Repeatability estimates remained above 0.78 for this trait across all analyzed DIM. Repeatability estimates for EB showed more variation, ranging from 0.42 to 0.65, with the highest values found at the beginning of lactation. Repeatability estimates of DMI were also moderate and ranged from 0.41 to 0.56.

For each single DIM (11–180), the highest heritability was found for ECM ($h^2 = 0.58$ – 0.68) and the lowest heritability was found for DMI ($h^2 = 0.26$ – 0.37).

Figure 2. Trend of the mean and SD across DIM 11 to 180 for (a) ECM (in kg), (b) BW (in kg), (c) DMI (in kg), and (d) energy balance (EB; in MJ of NE_L).

Figure 3. Heritability and repeatability estimates for energy balance (EB), DMI, and ECM across the first 180 DIM. Lines represent daily estimates obtained from random regression analyses. Approximate SE of heritability estimates ranged from 0.03 to 0.05, 0.02 to 0.04, and 0.04 to 0.04 for EB, DMI, and ECM, respectively. Approximate SE of repeatability estimates ranged from 0.01 to 0.02, 0.01 to 0.02, and 0.00 to 0.01 for EB, DMI, and ECM, respectively.

The heritability estimates of EB ranged between 0.29 and 0.49 and showed a similar pattern (i.e., a slightly parabolic curve as the heritability for DMI; Figure 3). The heritability of EB is intermediate between the heritabilities of the other traits throughout the entire interval, which can be expected as DMI and ECM are the key determinants of EB.

As discussed earlier (Tetens et al., 2014), the mean heritability for DMI across the first 180 DIM was in general agreement with other studies, although some authors (Berry et al., 2007; Spurlock et al., 2012) found different trajectories. In a more recent study, however, Li et al. (2016), who also analyzed DMI in primiparous Holstein cows across the first 180 DIM, reported a very similar trajectory, also with a similar mean heritability of approximately 0.3. For EB, the overall dimension of heritability estimates is also in general agreement with previous studies, although the variation of estimates reported in the available literature is rather high. The heritabilities estimated for ECM in the current study seem high, with some values close to 0.7. This is likely attributable to the study being conducted in a single well-managed research herd. This has also been found by Byskov et al. (2017), who compared a research herd

with commercial farms. For ECM in the research herd, they found similar heritabilities as in the current study, whereas the estimates were much lower for commercial farms.

The estimates of the chip heritabilities showed a slightly different trajectory across the 180 DIM (see Supplemental Figure S1, <https://doi.org/10.3168/jds.2018-15480>). For ECM, chip heritability averaged 0.49, with the highest value of 0.5 at DIM 11 and a slight decrease toward mid lactation. The mean chip heritability values for EB and DMI were 0.41 and 0.37, respectively, with a local minimum around DIM 40. The heritability estimates for EB and DMI obtained from the random regression models approached each other toward DIM 180, whereas the chip heritabilities diverged. However, both estimates show that the heritability changed across lactation.

Genetic Correlations Within Traits Across DIM 11 to 180

All traits under consideration are influenced by various biological processes, and there is some evidence that the major determinants of EB have a different

genetic background across lactation stages (Strucken et al., 2015). Therefore, genetic correlations of EB, DMI, and ECM were estimated between different lactation days (DIM 11 and every 10th day from DIM 20 to 180). Genetic correlations between EB estimates from different lactation stages were all positive but inversely related to the length of the interval between compared DIM, decreasing from 1.00 to 0.37 (Figure 4a). The same is true for DMI; however, genetic correlations between DMI in early and mid lactation decreased more rapidly, reaching a minimum of 0.32 (Figure 4b). For ECM, genetic correlations remained above 0.59 (Figure 4c), suggesting that the genetics of ECM are less variable during lactation than those of DMI and EB. The approximate standard errors of the genetic correlations within a trait were generally low, ranging from 0.00 to 0.07 for EB, 0.00 to 0.08 for DMI, and 0.00 to 0.04 for ECM.

Our analyses are limited to the first 180 DIM, and thus no conclusions can be drawn regarding the correlations between early or mid and late lactation. The lower genetic correlations between lactation stages, however, indicate that selection aiming to change the trajectory of EB should focus on feed intake rather than on milk yield because there will be different directions of selection in early and mid or late lactation. However, inclusion of DMI in the breeding goal would then require repeated measurements of feed intake (Liinamo et al., 2012) or the availability of suitable proxies (e.g., derived from mid-infrared spectra routinely available from milk analysis; Shetty et al., 2017; Wallén et al., 2018).

Genetic and Genomic Correlations Between Traits at the Same DIM

Genetic correlations between ECM and DMI were all positive, ranging from 0.09 to 0.36, with the lowest values found at DIM 11 and the highest values found at DIM 180. As expected, ECM and EB were negatively correlated across the whole observation period (i.e., early and mid lactation; $r_g = -0.26$ to -0.59). The strongest relationship was found at the beginning of lactation, indicating that selection for increased milk yield in this stage will dramatically intensify the energy deficit postpartum. To address this risk, breeding for a higher DMI in the initial lactation can represent a suitable strategy because the relationship between DMI and EB was more pronounced ($r_g = 0.71$ – 0.81) compared with the correlation between ECM and EB. There is only a weak correlation between DMI and ECM at the beginning of lactation, which increases to 0.36 in mid lactation. This is in contrast with other studies (Manzanilla Pech et al., 2014; Li et al., 2018)

Figure 4. Genetic correlations across DIM for (a) energy balance (SE ranged from 0.00 to 0.07), (b) DMI (SE ranged from 0.00 to 0.08), and (c) ECM (SE ranged from 0.00 to 0.04).

reporting much higher correlations in mid lactation. Overall, the results indicate that selection for milk yield might lead to a correlated selection response in feed intake in later lactation, whereas no such effect can be expected in early lactation, when this would be very desirable. On the other hand, it might lead to an unwantedly high increase in feed intake in late lactation. The latter can, however, not be evaluated from our data because the data are limited to the first 180 DIM. Negative genetic correlations between feed intake and milk yield as reported by other authors (Veerkamp and Thompson, 1999; Karacaören et al., 2006; Manzanilla Pech et al., 2014) were not found in the current study. The estimates of genomic correlations obtained from the polygenic effect in the null model for the bivariate GWAS were, as expected, in almost perfect concordance with the r_g estimates from the random regression models (see Figure 5 and Supplemental Figure S2, <https://doi.org/10.3168/jds.2018-15480>).

Genome-Wide Association Analyses

The results of the genome-wide association analyses are presented in Figures 6 and 7 (for animated and color versions, see Supplemental Figures S3 to S8, <https://doi.org/10.3168/jds.2018-15480>) as well as Table 2. In total, 10 genome-wide significant associa-

tions were detected involving 4 traits or combinations of traits and 3 SNP markers located on 3 chromosomes. No genome-wide significant hits were found in the univariate analysis of DMI (Figure 6b, Table 2), which is consistent with previous results (Tetens et al., 2014). From Figures 6 and 7, it becomes obvious that trait associations are clearly lactation stage specific, with a peak at a particular DIM; only some markers show associations over a larger lactation interval (e.g., the association signal for EB on BTA16; see Figure 6a). These results are in line with the reported genetic correlations among lactation stages and emphasize the varying genomic architecture of energy metabolism across lactation, which has to be considered when incorporating this trait complex into a breeding goal. Most of the association results can be ascribed to only a few genomic regions that exhibit effects on different traits. Table 2 summarizes these regions, including all markers associated at a relaxed P -value threshold of $P \leq 10^{-5}$, and shows their respective effects. Within this table, each association was assigned to a putative QTL according to the position of the SNP marker and the DIM (e.g., 2 signals were considered as belonging to the same QTL if they are located in the same chromosomal region and exhibit their effect within the same lactation stage). Based on this definition, 6 QTL were identified in the univariate analyses, 3 of which were also found

Figure 5. Genetic correlations between energy balance (EB), DMI, and ECM with approximate SE in parentheses across the first 180 DIM estimated from random regression models.

in bi- and multivariate runs. Six additional QTL were detected only in bi- and multivariate analyses.

Two QTL, namely numbers 4 and 9, were consistently mapped across more than 2 different GWA runs (Table 2). The QTL 4 is located around 36 Mbp on BTA16 and exhibits effects toward the end of the analyzed lactation period. In the univariate analysis, a negative effect on EB was estimated and was consistently found in the bivariate analyses involving EB as well. The signs of the effect estimates for DMI and ECM in these analyses are negative and positive, respectively,

which is in accordance with the results from the bivariate analysis involving these latter 2 traits. Generally, a QTL influencing EB can act on energy input (i.e., basically DMI), energy output (i.e., basically ECM), or energy allocation (e.g., reflected by body fat mobilization or deposition; Puillet et al., 2016). The latter could be evaluated only by considering weight change as a trait, which was, however, not done here. The QTL 4 most likely affects EB by exhibiting adverse effects on ECM and DMI. The simultaneous analysis of EB and its key determinants DMI and ECM thus provides

Figure 6. Three-dimensional Manhattan plots depicting the results of univariate genome-wide association analyses. The x- and y-axes represent genomic coordinate (UMD 3.1; https://oct2018.archive.ensembl.org/Bos_taurus/Info/Annotation) and negative decadic logarithm of the P -value, respectively. The z-axis represents lactation day. Only results with $-\log_{10}(P) \geq 4$ are shown; larger dots indicate genome-wide significant associations. EB = energy balance. For a better visualization, animated versions of the plots can be found in Supplemental Figures S3 to S5 (<https://doi.org/10.3168/jds.2018-15480>).

Figure 7. Three-dimensional Manhattan plots depicting the results of bi- and multivariate genome-wide association analyses. The x- and y-axes represent genomic coordinate (UMD 3.1; https://oct2018.archive.ensembl.org/Bos_taurus/Info/Annotation) and negative decadic logarithm of the P -value, respectively. The z-axis represents lactation day. Only results with $-\log_{10}(P) \geq 4$ are shown; larger dots indicate genome-wide significant associations. EB = energy balance. For a better visualization, animated versions of the plots can be found in Supplemental Figures S6 to S8 (<https://doi.org/10.3168/jds.2018-15480>).

Table 2. Overview of the results from uni-, bi-, and multivariate genome-wide association analyses¹

Trait ²	QTL no. ³	Chr ⁴	SNP ⁵	Position ⁶	DIM with $P < 10E-5^7$	Direction of effect ⁸
EB	1	1	ARS-BFGL-NGS-34353	39,813,886	DIM 130–155	↑
	2	10	Hapmap47826-BTA-107963	15,974,499	DIM 120–180 (160–175)	↓
	3	15	ARS-BFGL-NGS-32125	27,905,645	DIM 11–45	↑
	4	16	ARS-BFGL-BAC-35294	36,284,584	DIM 140–180	↓
DMI	—	—	—	—	—	—
ECM	5	16	ARS-BFGL-NGS-26559	36,817,218	DIM 11–30	↑
	6	19	ARS-BFGL-NGS-114474	61,166,436	DIM 35–70	↓
bv.EB.DMI	7	2	BTB-01792501	16,527,366	DIM 11–15	EB: ↓, DMI: ↑
	5	16	ARS-BFGL-NGS-26559	36,817,218	DIM 35–50	EB: ↓, DMI: ↓
	4	16	ARS-BFGL-BAC-35294	36,284,584	DIM 180	EB: ↓, DMI: ↓
	8	X	BTA-105578-no-rs	51,234,785	DIM 110–150	EB: ↓, DMI: ↓
bv.EB.ECM	9	1	ARS-BFGL-NGS-22536	80,269,992	DIM 45–60	EB: ↑, ECM: ↓
	9	1	BTB-00037326	81,406,451	DIM 40–120 (60–75)	EB: ↓, ECM: ↓
	10	1	ARS-BFGL-NGS-22608	100,481,800	DIM 11–25	EB: ↓, ECM: ↓
	3	15	ARS-BFGL-NGS-32125	36,817,218	DIM 11–50	EB: ↓, ECM: ↓
bv.DMI.ECM	11	16	ARS-BFGL-NGS-26559	27,905,645	DIM 15–25	EB: ↓, ECM: ↓
	4	16	ARS-BFGL-BAC-35294	36,284,584	DIM 170–180	EB: ↓, ECM: ↓
	12	28	BTB-01171367	21,292,836	DIM 110–155	EB: ↓, ECM: ↑
	9	1	ARS-BFGL-NGS-22536	80,269,992	DIM 35–75	DMI: ↓, ECM: ↓
mv.EB.DMI.ECM	9	1	BTB-00037326	81,406,451	DIM 45–120	DMI: ↓, ECM: ↓
	4	16	ARS-BFGL-BAC-35294	36,284,584	DIM160–180 (180)	DMI: ↓, ECM: ↓
	9	1	BTB-00037326	81,406,451	DIM 45–95 (65)	EB: ↑, DMI: ↓, ECM: ↓
	3	15	BTB-00587441	25,693,584	DIM 40–80	EB: ↓, DMI: ↓, ECM: ↑

¹The table contains all trait-associated markers with a P -value below the arbitrarily chosen threshold of $10E-5$.
²EB = energy balance. The prefixes bv. and mv. indicate bi- and multivariate analyses, respectively.
³The same QTL number was assigned to all associated markers falling into the same 3-Mbp window and showing associations for overlapping or adjacent lactation stages.
⁴Cattle chromosome.
⁵Illumina SNP identifier.
⁶Position in base pairs according to genome version UMD 3.1 (https://oct2018.archive.ensembl.org/Bos_taurus/Info/Annotation).
⁷All DIM with a $-\log_{10}(P) \geq 5$ for that particular SNP; DIM with genome-wide significant association results are given in parentheses.
⁸Direction of effects estimated for the rare allele. Positive effects indicate a higher DMI, a higher milk yield, and a more positive EB.

mechanistic insights with respect to possible underlying genes. Screening the QTL region on BTA16 for possible candidate genes revealed the gene encoding fumarate hydratase to be located only 200 kb apart from the best associated SNP. This enzyme, also termed fumarase, belongs to the Krebs cycle, which could be viewed as the “turntable” of energy metabolism. From this point of view, it might be a promising candidate gene. In humans, genetic variation in this has been mainly reported to be involved in the development and progression of cancer (Sajjani et al., 2017) as well as severe fumarase deficiency syndromes (Ryder et al., 2018). The activity of fumarase in the nucleus accumbens has been described as a biomarker for addiction and eating disorders in humans (Alguacil et al., 2011). It has also been related to palatable food seeking in mice (Pérez-Ortiz et al., 2017), and upregulation in the brain has been observed as a consequence of food deprivation in rats (Lizárraga-Mollinedo et al., 2013).

The QTL 9 is located on BTA1 between 80 and 82 Mbp and consistently shows up as associated around DIM 65 in the bivariate analyses for EB and ECM and for DMI and ECM as well as in the multivariate analysis incorporating all 3 traits, but not in univariate analyses. As for QTL 4, the estimated SNP effects for EB and ECM (Table 2) have different signs, whereas those for DMI and ECM have the same sign. The effect estimate for ECM is, however, roughly 2.5 times larger than that for DMI, which explains the effect on EB. We also screened the respective genome regions for candidate genes and found the adiponectin-encoding gene *ADIPOQ* to be located only approximately 400 kbp apart from the best associated marker BTB-00037326. Adiponectin, which is secreted by adipocytes, exerts insulin-sensitizing effects, and in obesity-linked insulin resistance it has been shown to be downregulated along with its receptors (Caselli, 2014). In dairy cows, positive associations between serum adiponectin levels and insulin responsiveness of the glucose and fatty acid metabolism have been established, whereas a negative association has been reported with BCS during the dry period (De Koster et al., 2017). Other studies reported the negative influence of high NEFA levels on feed intake, where adiponectin also plays a regulatory role (Kuhla et al., 2016). Interestingly, a variable duplication in the *ADIPOQ* promoter was reported to be associated with marbling, rib-eye area, and fat thickness in beef cattle (Zhang et al., 2014).

CONCLUSIONS

Based on longitudinal and multivariate analyses of EB, DMI, and ECM across the first 180 DIM, we were able to demonstrate a clearly lactation stage-specific

genetic architecture of energy homeostasis with heritability estimates and genetic correlations that varied in the course of lactation and DIM- or lactation stage-dependent association signals. It seems possible to optimize the lactation trajectory of especially DMI to improve animal health in early lactation and efficiency in later lactation. Based on GWAS results, we identified the genes encoding fumarate hydratase and adiponectin as possible candidates. However, studies with larger cohorts, ideally at the sequence level and including additional traits such as weight change or metabolomics data, will have to be conducted in the future to confirm and refine these results.

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