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Genetic Architecture and Biological Basis of Feed Efficiency in Dairy Cattle

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ABSTRACT: The genetic architecture of residual feed intake (RFI) and related traits was evaluated using a dataset of 2,894 cows. A Bayesian analysis estimated that markers accounted for 14% of the variance in RFI, and that RFI had considerable genetic variation. Effects of marker windows were small, but QTL peaks were identified. Six of the 8 chromosomes harboring QTL influencing RFI did not contain QTL influencing dry matter intake (DMI), net energy for lactation, or metabolic body weight. In contrast, 7 of 9 chromosomes with QTL influencing DMI also harbored QTL for one or more of the other traits evaluated. These results represent the first genomic analysis of RFI using a large (~3,000 animals) international dataset. In general they suggest RFI is a trait that should respond to selection, and that its genetic regulation is different from that of DMI.
Keywords: dairy cattle**;** feed efficiency**;** genome wide association study

Introduction

Feed costs for U.S. dairy operations have traditionally accounted for approximately 50% of the total farm budget, yet this percentage is increasing due to increased competition for feed ingredients. Additionally, the dairy industry is working to improve environmental stewardship while maintaining an adequate supply of dairy products for a growing human population. Consequently, improving the efficiency with which dairy cows convert feed to milk is a critical issue. Current estimates of genetic parameters for traits related to feed efficiency, including dry matter intake (DMI) and residual feed intake (RFI), indicate these traits should respond to selection pressure for improved feed efficiency. Additionally, genomic prediction will likely facilitate the incorporation of this difficult to characterize trait into industry- wide selection indexes. However, the biological basis and genetic architecture underlying the regulation of feed efficiency in lactating dairy cows are not well understood.

Feed efficiency is a complex trait determined by several component traits including the production of milk and milk components, feed intake, maintenance requirements, and change in body energy reserves. Genetic selection strategies for improving feed efficiency include selection for decreased DMI, and selection for animals that consume less feed than expected based on their energy requirements, i.e. negative RFI. Understanding the genetic architecture underlying the regulation of these component traits will aid in understanding the biological regulation of feed efficiency and the potentially complex regulation of RFI. Specifically, genes that influence the efficiency with which cows convert feed to milk by regulating biological processes other than feed intake, production, and body size traits may not be targeted by selection based on component traits alone. Additional physiological processes such as heat loss, digestibility, and energy partitioning contribute to differences in feed efficiency in some species. Selection for these underlying component traits may be minimal if selection is based on a combination of DMI, production and body size traits rather than on selection for RFI as a more direct measurement of feed efficiency. Comparison of the underlying genetic architecture of traits related to feed efficiency, including RFI, may help to elucidate optimal selection strategies, as well as biological pathways that contribute to variation in these traits.

The objective of this research was to characterize the genetic architecture regulating RFI, DMI, maintenance energy requirements, change in body energy reserves, and net energy used for milk production. Candidate genes within quantitative trait loci (QTL) regions were identified based on current knowledge of their biological function.

Materials and Methods

Data. The data used in this study were collated from multiple experiments conducted throughout the U.S., Scotland, and the Netherlands. Phenotypes were recorded for daily milk yield (kg), DMI (kg), milk fat, protein and lactose percent, and body weight (kg). Genotypes were determined using the BovineSNP50 BeadChip (Illumina). A total of 2,894 cows, including 1,645 from the U.S., 797 from the Netherlands, and 452 from Scotland were used in analyses. Of these, 1,870 were primiparous and 1,024 were multiparous cows. Each cow was represented by a single parity in the final analyses.

Calculation of RFI. A single RFI phenotype for each cow was calculated based on the mean of records representing the first 28 day period with both milk and DMI records, between 50 and 200 days in milk. Dry matter in-

take was modeled as a linear function of key energy sinks plus fixed and random effects as follows:

$$DMI = \mu + fixed\ effects + random\ effects + b_1 NE_L + b_2 MBW + b_3 dBW + RFI \quad [1]$$

Here μ represents the overall mean; fixed effects include parity class (primi or multiparous), a 5th order polynomial on days in milk, and the interaction between the two; random effects included month of records and experimental treatment; net energy for lactation (NE_L) was derived from milk, fat, protein, and lactose yields as defined in NRC (2001); maintenance energy requirements, represented as metabolic body weight (MBW), were defined as body weight (kg) to the power of 0.75; and change in body weight (dBW) was the average daily change in body weight (kg) over the 28 d period. The b_1, b_2, and b_3 represent partial regression coefficients on the corresponding energy sinks, and RFI is the estimated residual of the model. Separate analyses were run using data from each country.

Genome Wide Association Study. Genotypes were transferred to the USDA's Animal Improvement Programs Laboratory (AIPL) where they were evaluated for quality and pedigree conflict as previously described (Wiggans et al. (2011)). Genotypes were imputed to a final set of 61,013 SNPs. This SNP set includes all SNP traditionally used in U.S. genomic evaluations, plus an additional 15,818 selected from higher density chips based on the magnitude of their effect on traits evaluated in the U.S. (Wiggans et al. (2014)).

Analyses for the genome wide association study (GWAS) were carried out using GenSel software (Fernando and Garrick (2009)). Genetic parameters were estimated using the BayesCPi option. Traits of interest included DMI and the four primary energy sinks shown in [1]; NE_L, MBW, dBW, and RFI. For GWAS analysis, dBW was expressed as the net energy associated with change in body weight gain adjusted for body composition (NEg) such that

$$NEg = (3 + Body\ Condition\ Score) * dBW \quad [2]$$

Models for DMI, NE_L, MBW, and dBW included parity class (primi or multiparous) and cohort group, defined to represent treatments within experiments within research station. Small cohorts within research station were combined such that each cohort had a minimum of 8 animals. Days in milk was also included in the analysis as a linear covariate. Fixed and random effects were omitted from analyses of RFI as these effects had been accounted for in the calculation of RFI. Final GWAS analyses were performed using the BayesB option of GenSel, with pi equal to 0.99. Genetic and residual variances estimated from the BayesCPi analyses for each trait were used as starting values for the BayesB analyses. In addition to individual SNP effects, 1 Mb windows were constructed across the genome and the cumulative effects of markers within windows were estimated. The 10 windows with greatest effects for each trait were further investigated to identify regions with pleiotropic effects and potential positional candidate genes.

All annotated genes located within the 10 windows with greatest effect on RFI were retrieved, and functional pathway clustering was performed using DAVID (Dennis et al. (2003)).

Results and Discussion

Genetic Parameters. Estimates of genetic variance, proportion of phenotypic variance accounted for by SNP, and Pi resulting from the BayesCPi analyses are shown for each trait in Table 1. The percent of variance in DMI, NE_L and MBW accounted for by markers is consistent with previous estimates from this and other datasets (Spurlock et al. (2012); Berry et al. (2014)). A similar non-significant heritability was found for GAIN when we evaluated a similar dataset using a traditional pedigree analysis (unpublished data). Because of this non-significant heritability estimate, this trait was not considered further. Our group is the first to investigate genetic parameters for RFI using a large (~3,000 animals) dataset that combines data from multiple studies across multiple countries. Similar to a previous pedigree analysis (Tempelman et al., in preparation), the current marker heritability estimate indicates RFI to be a low to moderately heritable trait such that RFI would respond to genetic selection. Importantly, animals with extremely high and low genetic values (differing by ±4 genetic standard deviations) for RFI are expected to differ by approximately 2.0 kg of DMI per day. If this difference is maintained over 305 days of a lactation, and the cost of feed is $0.25 per kg, this represents a difference of approximately $153 in feed costs per lactation from cows with the lowest compared to highest genetic value for RFI.

Table 1. The overall mean, estimates of genetic variance (VarG), proportion of phenotypic variance accounted for by SNP (Mh^2), and Pi, such that 1-Pi represents the proportion of SNP fitted in the genome wide association analyses, for traits related to feed efficiency.

Trait[a]	Mean	VarG	Mh^2	Pi
DMI	21.8	1.54	0.26	0.93
NE_L	26.9	3.27	0.22	0.91
MBW	118.9	22.50	0.38	0.92
NEg	0.39	0.17	0.02	0.98
RFI	0	0.27	0.14	0.91

[a]DMI = dry matter intake (kg/d); NE_L = net energy for lactation (MCal/d); MBW = metabolic body weight representing maintenance energy requirements (MCal/d); NEg = net energy associated with change in body weight gain adjusted for body composition (MCal/d); RFI = residual feed intake (kg/d).

Genetic Architecture for Traits Related to Feed Efficiency. The relative location and magnitude of effects from 1 Mb windows are presented as Manhattan plots for RFI, DMI, NE_L, and MBW in Figures 1 through 4. In general, individual effects were small, explaining less than 2% of total genetic variance. This result likely reflects the complexity of the traits, and is similar to that previously reported for a similar study (Veerkamp et al., (2012)). Because of the small magnitude of effects, we arbitrarily chose the 10 windows with greatest effect on each trait for further review (Table 2).

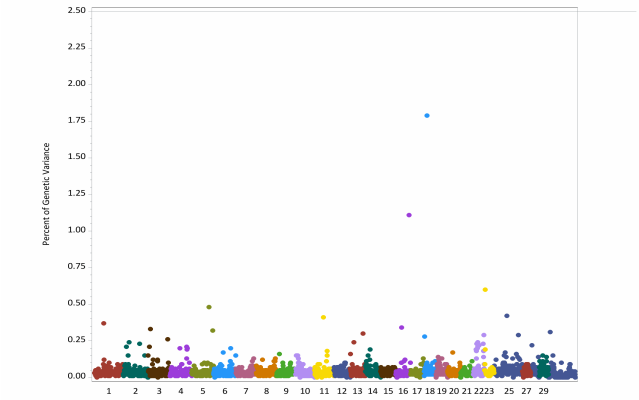

Figure 1. Genetic architecture of Residual Feed Intake from a genome wide association study of data from 2,894 cows and a panel of 61,013 SNP presented as the percent of genetic variance explained by 1-Mbp windows across the genome.

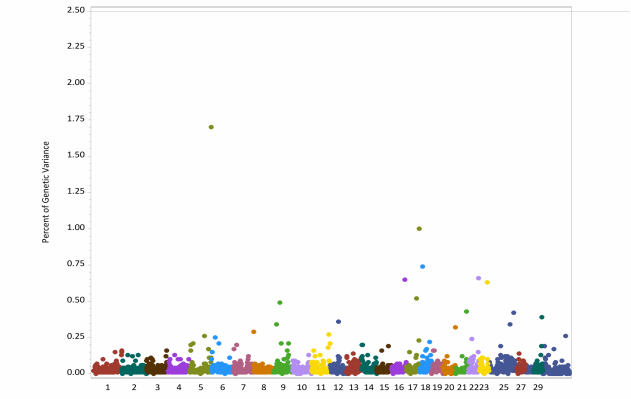

Figure 2. Genetic architecture of Dry Matter Intake from a genome wide association study of data from 2,894 cows and a panel of 61,013 SNP presented as the percent of genetic variance explained by 1-Mbp windows across the genome.

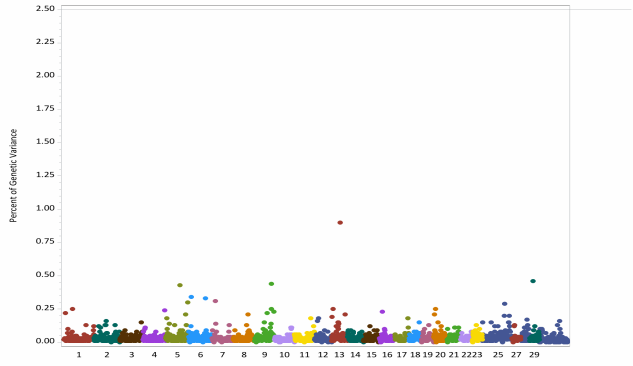

Figure 3. Genetic architecture of Net Energy for Lactation from a genome wide association study of data from 2,894 cows and a panel of 61,013 SNP presented as the percent of genetic variance explained by 1-Mbp windows across the genome.

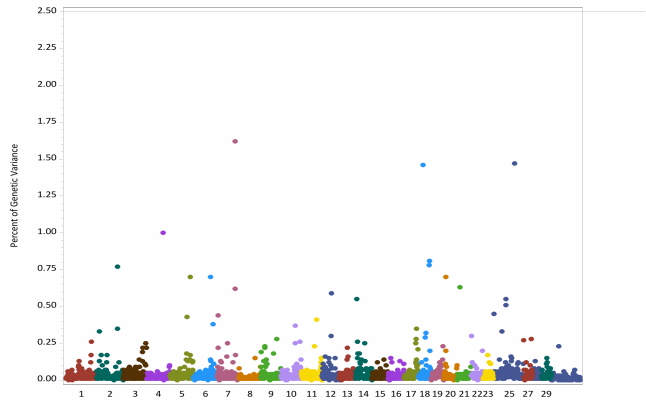

Figure 4. Genetic architecture of Metabolic Body Weight from a genome wide association study of data from 2,894 cows and a panel of 61,013 SNP presented as the percent of genetic variance explained by 1-Mbp windows across the genome.

Table 2. The 10 chromosomal regions (1-Mbp windows) with the greatest effects on dry matter intake (DMI), net energy for lactation (NE_L), metabolic body weight (MBW) and residual feed intake (RFI) including the chromosomal location, estimate of percent of genetic variance explained (PERCENT), and relative ranking for each trait is shown for each window.

Chr.	Mbp	PERCENT	DMI	RFI	NE_L	MBW
1	51	0.37		7		
2	109	0.77				7
3	12	0.33		9		
4	4	0.39	10			
4	85	1.00				4
5	75	0.43			4	
5	95	0.48		4		
5	105	0.70				8
5	116	0.30			8	
5	117	0.32		10		
5	121	2.07	1			

6	13	0.34			5	
6	88	0.33, 0.70			6	10
7	18	0.31			7	
7	92	1.62				1
9	73	0.46	6			
9	88	0.44			3	
11	48	0.41		6		
13	46	0.90			1	
16	32	0.34		8		
16	73	1.11		2		
17	56	0.45	7			
17	72	0.55	4			
18	15	0.71, 1.79	2	1		
18	23	1.46				3
18	56	0.78				6
18	57	0.81				5
20					10	0.28
20	12	0.70				9
20	70	0.44	8			
21	59	0.50	5			
22	56	0.66	3			
23	4	0.60		3		
25	8	0.42		5		
26	4	1.47				2
26	12	0.29			9	
29	18	0.46			2	
29	47	0.40	9			
X	1	0.53	5			

The top 10 QTL influencing RFI were found on a total of 8 chromosomes. Six of these 8 chromosomes did not harbor QTL influencing other traits evaluated in this study. This result is expected if RFI is independent from NE_L and MBW at the genetic level, and is consistent with very low genetic correlations estimated between RFI and these traits (VandeHaar et al. (2014)). Furthermore, it may be speculated that these QTL impact feed efficiency by altering physiological pathways that affect cellular use of energy and ATP, rather than the partitioning of energy toward milk production or maintenance. When considering the 2 chromosomes that harbored QTL influencing RFI and additional traits, *Bos taurus* (BTA) 18 contained a QTL with pleiotropic effects on both RFI and DMI. This result is expected if the effect of the QTL on RFI is large enough that it also accounts for a significant portion of variation in DMI. This was the QTL with the largest effect on RFI, and may be the only region to have a large enough effect on RFI to also be detected as a QTL influencing DMI. Three of the top 10 windows influencing MBW were also found on BTA18. The window with the largest effect on MBW was located 8 Mbp from the RFI/DMI QTL, while the other 2 windows were more distal (41-42 Mbp). The final QTL influencing RFI was on BTA5, in a general region that also harbored QTL influencing DMI, NE_L, and MBW.

In contrast to RFI, 7 of 9 chromosomes with QTL influencing DMI harbored QTL influencing one or more of the energy sink traits we evaluated (NE_L, MBW or RFI). This result is consistent with the hypothesis that a portion of variation in DMI is associated with variation in each of the energy sinks, as reflected by relatively high genetic correlations between DMI and NE_L (0.73) and between DMI and MBW (0.40; unpublished results). For example, the QTL influencing DMI on BTA5 had the greatest effect of all QTL on DMI. Additional QTL influencing NE_L, MBW, and RFI were all found within 20 Mbp of the DMI QTL. Other examples of co-regulation of DMI and energy sink traits by QTL within 30 Mbp were found on BTA9 (DMI and NE_L), 18 (DMI, RFI and MBW), and 29 (DMI and NE_L), while QTL influencing DMI and MBW, and DMI, NE_L, and MBW separated by more than 50 Mbp were found on BTA4 and 20, respectively.

Comparison with QTL Identified in Prior Studies. The greatest effect on RFI was found for a window located on BTA18. This region had a pleiotropic effect on DMI, and multiple windows influencing MBW were also identified on BTA18. Veerkamp et al. (2012) completed a GWAS analysis using data from 1,629 cows, including approximately 950 cows shared with the current study. A SNP on BTA18 (20.7 Mbp) was significantly associated with DMI, but explained only 0.01% of total variance. Also similar to the current study, SNP associated with live weight were identified more distally on BTA18 (57-58 Mbp). Yao et al. (2013) analyzed a subset of the data from the current study using a novel random forest approach. Multiple SNP on chromosome 18 were associated with RFI, as 6 of the 25 top ranking SNP from the random forest analysis were from this chromosome. Additionally, 2 studies of RFI in beef cattle noted significant QTL on BTA18 in a region similar to that identified in the current study (Sherman et al. (2009); Barendse et al. (2007)). Several studies in dairy cattle reported a QTL on BTA18 impacting calving traits (Kuhn et al. (2003); Holmberg and Andersson-Eklund, (2006); Thomasen et al. (2008)) and conformation traits and live weight (Cole et al., (2009); Veerkamp et al., (2012)). Cole et al. (2009) suggested the presence of a major pleiotropic QTL influencing calf birth weight and conformation traits on BTA18. This QTL was centered at 57.59 Mbp at a SNP located within an intron of the *Siglec-5* gene (Cole et al. (2009)). Together, data from the current study and recent literature define a QTL influencing conformation traits, including MBW, on BTA18 near 57 Mbp. The current study and previous studies in dairy and beef cattle provide evidence for an additional QTL influencing DMI and RFI in the region of 15-25 Mbp on BTA18.

Other common regions influencing RFI were also found when comparing the top 10 windows from this study to results from previous studies. Five of the 8 top chromosomal regions identified in the current study were also represented among 188 markers with significant effect described by Yao et al. (2013). For the remaining regions, significant markers were identified by Yao et al. in other regions of the chromosome (Table 3). It was previously noted that similar chromosomal regions influencing RFI were identified by Yao et al. (2013) and Sherman et al. (2009) in dairy and beef cattle, respectively. Comparisons to the current results indicate similar chromosomes have

been identified across studies, but specific regions on those chromosomes are not consistent, with the exception of BTA18 (Table 3). However, the beef cattle studies utilized smaller numbers of animals and markers, and may have limited resolution of chromosomal regions compared to the current study. Many common QTL regions were identified across the current study and that of Veerkamp et al. (2012; Table 4). However, the current study also identified novel QTL that may reflect the influence of U.S. cattle in the current data set but not the former.

Table 3. Comparison of QTL influencing RFI in the current study the dairy[1] and beef[2,3,4] cattle literature. The location is shown (chromosome:Mbp) for QTL influencing RFI across studies.

Current Study	Yao[1]	Barendse[2]	Sherman[3]	Rolf[4]
1:51	1:41-146	1:1.4	1:5	1:85-133
3:12	3:86		3:82	3:7, 70
5:95, 117	5:105-108			5:36
11:48	11:36-68	11:90.0		11:40-105
16:32, 73	16:30-70			16:14
18:15	18:34-38	18:24.7	18:71-108	18:52
23:3	23:45	23:39.6	23:13	23:33
25:5	25:41	25:38.5		25:14-23

[1]Yao et al., 2013; 188 SNP representing all chromosomes
[2]Barendse et al., 2007; 34 regions representing all chromosomes
[3]Sherman et al., 2009; 18 regions representing 17 chromosomes
[4]Rolf et al., 2012; 65 SNP representing 27 chromosomes

Table 4: QTL regions in common (± 5 Mbp) between current study and Veerkamp et al., 2012 (Chromosome:Mbp).

DMI	Body Weight[1]	Adjusted Milk[2]
4:4	7:92, 93	5:75
5:121	18:57	20:10
18:15	20:12	
26:4		

Putative Candidate Genes. A total of 93 Ensembl gene identifiers were retrieved from the top ten 1-Mbp windows influencing RFI. Using the lowest classification stringency, the Gene Functional Classification Tool (DAVID) identified 2 gene clusters. The first (enrichment score 0.69) included 3 genes (*H2afj, ORC6L*, and *HIST1H4D* from BTA5, 18, and 5, respectively) involved in nucleosome structure. The second cluster (enrichment score 0.44) included 6 genes (*Pkdrej, celsr1, TRAF5, EFCAB2, SMYD3 and Dnaja2* from chromosomes 5, 5, 16, 16, 16 and 18, respectively) involved in signal transduction.

In addition to these functional gene clusters, several individual putative candidate genes of interest were noted. Three genes related to mitochondrial function and translation were localized within the RFI QTL region on BTA11. Mitochondrial function has been identified as a critical factor influencing feed efficiency in multiple species (Spurlock and VandeHaar (2013)). The QTL region on BTA18 that had the largest effect on RFI included the gene for glutamic pyruvate transaminase, which is involved in amino acid metabolism and gluconeogenesis. These key metabolic pathways could be involved in the regulation of feed efficiency. Finally, the QTL region on BTA5 harbors the gene for PPAR alpha, a key regulator of lipid metabolism. Given the central role of lipid metabolism in milk fat synthesis, PPAR alpha is an intriguing candidate gene for the cluster of QTL on BTA5 that influenced all traits evaluated in this study. Additionally, a microarray study identified the PPARA pathway as a putative target of selection for divergent RFI in pigs (Lkhagvadori et al. (2010)).

Conclusion

Results demonstrate that significant genetic variation exists for RFI in lactating dairy cattle, and identifies genomic regions most closely associated with variation in RFI. The QTL with largest effect on RFI were rarely associated with other traits that account for energy utilization, suggesting these QTL may reflect biological pathways associated with efficiency of energy utilization. Comparison of RFI QTL across experiments investigating feed efficiency in beef and dairy cattle identified BTA1, 18, and 23 as consistently harboring QTL influencing RFI, although different regions of these chromosomes were sometimes cited. The identification of genes within the top RFI QTL regions revealed several putative candidate genes of particular interest, including genes involved in signal transduction, mitochondrial function, and lipid metabolism.

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