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Genome-wide associations for feed utilisation complex in primiparous Holstein–Friesian dairy cows from experimental research herds in four European countries

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Genome-wide association studies for difficult-to-measure traits are generally limited by the sample size with accurate phenotypic data. The objective of this study was to utilise data on primiparous Holstein–Friesian cows from experimental farms in Ireland, the United Kingdom, the Netherlands and Sweden to identify genomic regions associated with the feed utilisation complex: fat and protein corrected milk yield (FPCM), dry matter intake (DMI), body condition score (BCS) and live-weight (LW). Phenotypic data and 37 590 single nucleotide polymorphisms (SNPs) were available on up to 1629 animals. Genetic parameters of the traits were estimated using a linear animal model with pedigree information, and univariate genome-wide association analyses were undertaken using Bayesian stochastic search variable selection performed using Gibbs sampling. The variation in the phenotypes explained by the SNPs on each chromosome was related to the size of the chromosome and was relatively consistent for each trait with the possible exceptions of BTA4 for BCS, BTA7, BTA13, BTA14, BTA18 for LW and BTA27 for DMI. For LW, BCS, DMI and FPCM, 266, 178, 206 and 254 SNPs had a Bayes factor >3, respectively. Olfactory genes and genes involved in the sensory smell process were overrepresented in a 500 kbp window around the significant SNPs. Potential candidate genes were involved with functions linked to insulin, epidermal growth factor and tryptophan.

Keywords: genome-wide association, feed intake, cow, live-weight

Implications

Several single nucleotide polymorphisms (SNPs) were associated with live-weight, dry matter intake, body condition score and milk yield in a dataset combining data from research herds in four countries. In regions with significant SNP, potential candidate genes were identified for biological processes linked to taste and smell, a gene linked to the essential amino acid tryptophan that is involved with feeding motivation, as well as genes linked to regulation of insulin or epidermal growth factor.

Introduction

There has been long running interest in how feed intake and feed efficiency should be taken into account in breeding

decisions (see Veerkamp, 1998). Quantitative genetic studies have shown that genetic variation exists among animals in the feed utilisation complex, and in its component traits (e.g. feed intake, milk production and body weight; Veerkamp *et al.*, 2000; Berry *et al.*, 2007). Feed intake, as a trait, was initially targeted in breeding programs to try to reduce the quantity of feed required per unit of production, that is, improving feed efficiency. However, in the past two decades, interest has shifted towards the role of feed intake and its relationship with energy balance (EB), health and fertility. This shift in interest arose from the fact that current genetic selection for yield increases feed intake, but also results in a more negative energy balance (NEB) and more body tissue mobilisation during lactation (see Veerkamp *et al.*, 1993; Dillon *et al.*, 2006). As demonstrated by the size and direction of the genetic correlation between milk yield and feed intake and by the negative genetic correlation

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between milk yield and measures of EB, live-weight (LW; change) and body condition score (BCS; Veerkamp *et al.*, 2003), the expected correlated response in feed intake from selection on yield alone cannot cover the extra requirements needed for the increased yield. More recently, genetic selection for improvement in the feed utilisation complex has received increased interest as a means of reducing methane emission of dairy cows (Wall *et al.*, 2010).

Despite the reoccurring need to include the feed utilisation complex in dairy cattle breeding goals (i.e. improving feed efficiency, maintaining EB and/or reducing methane emission), there is, as yet, no direct selection practiced. This is primarily because of the large resource demand of measuring individual feed intake in dairy cows. This makes routine selection in breeding programmes too difficult, but also means that studies that estimated genetic parameters for the feed utilisation complex in dairy cows have generally been confined to a small number of animals from research herds (Svendsen *et al.*, 1994; Veerkamp *et al.*, 2000; Berry *et al.*, 2007). As a result, there has been considerable interest in predictors of the feed utilisation complex, like, for example, BCS (Koenen *et al.*, 2001; Banos *et al.*, 2004; Dal Zotto *et al.*, 2007), or other predictors of body energy state (Coffey *et al.*, 2003). However, studies with actual feed intake measures tend to have few animals recorded, which makes it difficult to establish the driving sources of genetic variation in feed efficiency (see Veerkamp *et al.*, 1993; Veerkamp, 2002) and optimal selection strategies due to lack of reliable genetic parameters.

Recent developments in genotyping technology have provided an alternative method of selection, in which DNA information is used. This would allow relatively cheap selection, compared with progeny testing for feed intake. Few studies have looked at the individual gene level for explaining genetic variation in feed intake. Examples are leptin, growth hormone or DGAT1 (Liefers *et al.*, 2002; Liefers *et al.*, 2005; Banos *et al.*, 2008; Oikonomou *et al.*, 2009), but the recent availability of fast-throughput genotyping platforms with dense genome-wide markers has reduced the cost of genotyping considerably, making whole-genome association studies a viable alternative. Potentially, functional processes and (quantitative trait loci) QTL that underlie the genetic variation in the feed utilisation complex might be identified. Nevertheless, accurate phenotypes on large numbers of animals and available in one dataset is the limiting factor in such association studies. To overcome this limitation, in this study we collated cow genotypic and phenotypic information from research herds in Ireland, the United Kingdom, the Netherlands and Sweden and related genetic markers across the genome to the phenotypes for the feed utilisation complex using a Bayesian Stochastic Search Variable Selection (BSSVS) statistical approach. The objective of this study was to identify putative genes and functional processes at gene level that might be involved in genetic variation in the feed utilisation complex. Fat-protein corrected milk yield is an important component of the feed utilisation complex, but has been given relatively little attention in this study, as much larger datasets are available in national programs.

Material and methods

The data used in the present study originated from Teagasc, Moorepark, Ireland; the Langhill herd from Scottish Agricultural College, United Kingdom; two herds of Wageningen UR Livestock Research (NBZ and GEN), the Netherlands and the Jälla herd of the Swedish University of Agricultural Science. A detailed description of the experimental treatments imposed on the animals in the different countries is provided elsewhere, for Scotland (Veerkamp *et al.*, 1995; Pryce *et al.*, 1999; Coffey *et al.*, 2004), Ireland (Horan *et al.*, 2005), Sweden (Petersson *et al.*, 2006), the Netherlands (Veerkamp *et al.*, 2000; Beerda *et al.*, 2007), and a more detailed description of the merging of the data sources and variance components across the different herds is given by Banos *et al.* (2012). The analyses by Banos *et al.* (2012) illustrated the benefits of combining data across herds, and therefore the Swedish data were added in this study to increase the number of records for milk yield.

Phenotypic data

Originally, phenotypic data were available on 2031 Irish, 1018 United Kingdom, 725 Dutch and 225 Swedish Holstein-Friesian cows. However, for this study only first lactation records were selected (Table 1), resulting in 1804 first lactation Holstein-Friesian cows with 66 116 test-day records collected up to 45 weeks (315 days) in lactation. Recorded traits were milk, fat and protein yield, LW, BCS, dry matter intake (DMI) and fat and protein corrected milk yield (FPCM). Extreme values for these traits (comprising <1%) were excluded from further analysis, criteria used were for milk yield between 1 and 70 kg/day, with fat and protein yield >0.025 kg/day, LW had a minimum value of 100 kg and DMI of 1 kg/day. A description of data recording systems and frequency of measurements on each farm is described in detail by Banos *et al.* (2012). Data originated from different herds with different management and different frequencies of data recording and phenotypic data were pre-adjusted for management effects. For this purpose, a test-day model was fitted with a random permanent environmental animal effect as a sixth order polynomial (no genetic relationships included), a fixed effect for the mean lactation curve (fourth order polynomial) and a deviation of this mean curve for 10 management groups generated as an interaction between farm, nutritional treatment and milking frequency (2× or 3× daily) combinations. Specific time-dependent random effects were fitted for year-month of milk test by management group (353 levels) and a specific treatment effect was fitted for experimental treatments during lactation for the cows in Ireland (81 levels). The model was fitted in ASReml (Gilmour *et al.*, 2009) and used to predict a full lactation curve for each cow. The average LW, BCS, DMI and FPCM of the predicted values for weeks 3 to 15 were used in the further analysis, for all animals with 10 or more observations in this period in the dataset. This period was chosen to maximise the number of cows with sufficient feed intake records, as a substantial proportion of the Dutch feed intake data used here was recorded till 100 days in milk only.

Table 1 The total number of cows with records, years of recording, management groups (HC, MC and LC, respectively; twice or three times a day milking (2 or 3, respectively), total number of weekly records for FPCM, LW, BCS and DMI separately and average number of weekly records for each cow per trait used in the pre-adjustment procedure

Country	Farm	No. of cows	Years of recording	Management groups	Total number of weekly records				Average number of weekly records per cow			
					FPCM	LW	BCS	DMI	FPCM	LW	BCS	DMI
UK	Langhill	558	1992 to 2010	HC2, LC2, MC2	17 526	19 709	18 531	14 892	31.4	35.3	33.2	26.7
NL	NBZ	89	2003 to 2004	HC2, HC3, LC2, LC3	1748	1825	623	1323	19.6	20.5	7.0	14.9
SE	SLU	209	1989 to 2009	HC2	7172	0	0	0	34.3	0.0	0.0	0.0
IE	Moorepark	415	1998 to 2009	LC2	16 814	13 487	6697	867	40.5	32.5	16.1	2.1
NL	't Gen	545	1991 to 1998	HC2	18 180	15 700	0	15 005	33.4	28.8	0.0	27.5

HC = high; MC = medium concentrate; LC = low concentrate; FPCM = fat and protein corrected milk; LW = live-weight; BCS = body condition score; DMI = dry matter intake; UK = United Kingdom; NL = the Netherlands; SE = Sweden; IE = Ireland.

Genotypic data

All animals with phenotypic information were genotyped with the Illumina BovineSNP50 BeadChip (Illumina Inc., San Diego, CA, USA) containing 54 001 single nucleotide polymorphisms (SNPs). SNPs that fulfilled the following criteria were included in the association study: (1) GenCall (GC)score >0.20 and GenTrain (GT)score >0.55; (2) call rate >0.95%; (3) minor allele frequency >0.01 in each country; and (4) no extreme deviation from Hardy–Weinberg Equilibrium (i.e. $\chi^2 < 600$). The GCscore and GTscore are quality measures on the genotype calls from the genotyping assay. After the quality control edits 37 590 SNPs remained. Checks for Mendelian inconsistencies between pedigree and SNP data were performed for all genotyped parent–offspring pairs and among sibs and animals with suspected erroneous pedigrees were removed (Calus *et al.*, 2011). After these edits, 1629 animals with phenotypes and genotypes remained in the data. Missing SNPs were imputed using Beagle (Browning and Browning, 2007). When chromosome number and location of a SNP is presented in this study, these are on the BovineSNP50 from UMD3.0 bovine genome assembly from the University of Maryland as provided by Illumina (July 2010; Illumina Inc.). To identify functional genes in proximity of the SNP, the R package BioMart (Haider *et al.*, 2009) was used based on the provided position on BTAU_4.0 (<http://www.ensembl.org>, Btau_4.0; April 2010). The package DAVID was used to cluster genes on functional process or gene function and to test whether certain processes and function were over-represented around the significant genes (Huang *et al.*, 2009b).

Linkage disequilibrium (LD)

The persistency of the LD across the genome for different subpopulations is important for the power of the genome-wide association study. Following de Roos *et al.* (2008), persistency of LD (r^2 and r) were investigated for nine subpopulations in the complete dataset. The UK animals were split into the Langhill selection and control line animals (UKS and UKC), in the Netherlands there were three groups being NBZ herd, and the selected and not selected animals at the GEN herd (GENS and GENC), the Swedish (SLU) and Irish animals (TEA) formed one group each, and also the genotypes

of 344 sires were used as an additional group (BULLS). It is important to note that all Irish animals were of North American ancestry. For each group, r and r^2 were calculated between all neighbouring SNP pairs following Hill and Robertson (1986). The correlation between populations for r was calculated across all marker distances. The decay in r^2 with marker distance was investigated by sorting on the distance between the neighbouring SNPs and taking the average r^2 and marker distance for bins of 50 SNPs. The average r^2 and distance were plotted and smoothed with a Loess Curve (smoothing parameter = 2/3) for each group. Pairs of SNPs closer together than 20 kbp were ignored, as many of these pairs had extremely low r^2 . Finally, to quantify the decay in r^2 with distance between the pairs, it was assumed that $r^2 \approx 1/(4N_e c + 1)$, where N_e is the effective population size and c is the distance in Morgan (Sved, 1971). The combined parameter $N_e c$ was estimated for each group using the nonlinear least-squares ('nls') in the R package 'stats', which enabled to compare N_e between the groups at a given genetic distance, albeit it is a very approximate estimate of the real N_e .

Variance component estimation

Genetic and residual (co)variances for the four traits defined above (LW, BCS, DMI and FPCM) were estimated using animal linear mixed models in ASReml (Gilmour *et al.*, 2009). No fixed effects were included in the model because pre-adjustments of the data were made before analysis. Pedigree information on each genotyped animal was traced back as far as possible; a total of 9368 identities over 19 generations were included in the pedigree file.

Genome-wide association analysis

The BSSVS model (described by Verbyla *et al.*, 2010) was used to sample whether SNPs were linked to a QTL or not. The model used was

$$y_i = \mu + a_i + \sum_{j=1}^n \sum_{k=1}^2 SNP_{ijk} + e_i$$

where y_i is the phenotypic record of animal i , μ is the overall mean, a_i is the random polygenic effect of animal i , n is

number of SNP markers, SNP_{ijk} is a random effect for allele k at locus j of animal i and e_i is a random residual for animal i . SNP effects (SNP_{ijk}) were estimated as $q_{ijk} \times v_j$ (Meuwissen and Goddard, 2004), where q_{ijk} is the size of the effect of allele k at locus j and v_j is a scaling factor for locus j . The vectors \mathbf{a} and \mathbf{e} were assumed to be normally distributed, $\mathbf{a} \sim \mathbf{N}(\mathbf{0}, \mathbf{A}\sigma_a^2)$ and $\mathbf{e} \sim \mathbf{N}(\mathbf{0}, \mathbf{I}\sigma_e^2)$, where \mathbf{A} is the numerator relationship matrix and \mathbf{I} is an identity matrix.

An indicator variable l_j was introduced into the hierarchical model to allow for scenarios where a limited number of SNPs with large effects exist and for other scenarios where a large number of SNPs all had an infinitely small effect (the infinitesimal model). This indicator variable takes a value of 1 or 0, depending on whether the SNP was included with a large effect in the model or not. The QTL-indicator l_j was sampled from

$$l_j | v_j, \sigma_v^2 \sim \text{Bernoulli} \left[\frac{\phi(v_j; 0, \sigma_v^2)(1 - \pi)}{\phi(v_j; 0, \sigma_v^2)(1 - \pi) + \phi\left(v_j; 0, \frac{\sigma_v^2}{100}\right)\pi} \right]$$

where π is the probability that l_j is unequal to 1, which follows a Bernoulli distribution. The probability that v_j is sampled from the distribution with large effects is $\phi(v_j; 0, \sigma_v^2)$ with variance σ_v^2 and mean 0. The prior QTL probability $(1 - \pi)$ used in the analyses had a value of 0.00027, reflecting the prior assumption that 10 QTL come from the distribution with large effects. This is equivalent to fixing π at a value of 0.99973, which is the term commonly used for BayesB type models to denote the proportion of SNPs that are *not* included in the model and therefore have zero effect, whereas for BSSVS it defines the proportion of loci with effects sampled from the distribution with small effects. The posterior distribution of l_j showed the proportion that the effects for locus j were sampled from the distribution with large effects, across all cycles after the burn-in. SNPs with the highest posterior probability were expected to be most likely linked to a QTL. A more detailed description of the model is given by Calus and Veerkamp (2011).

The estimated variance components from the quantitative genetic analysis were used to infer prior variances for the BSSVS model to estimate SNP effects. The BSSVS model was performed using Gibbs sampling, each time the model was run for 50 000 cycles with 10 000 cycles discarded for burn-in. For the prior QTL variance it was assumed that 80% of the genetic variance was explained by the SNPs and the remaining 20% by the polygenic component in the model. This division was based upon 80% of the total additive variance to be explained by the Bovine50 beadchip used in this study (Daetwyler, 2009). The 80% of the genetic variance explained by the SNP was split again across 10 SNPs sampled from the distribution with a large effect and the remaining 37 580 SNPs were sampled from the distribution with small effect (i.e. with a SNP variance that is one hundred times smaller than the variances for a SNP with a large

effect). Each trait was analysed with five different Gibbs chains of 50 000 cycles and average results of the five chains were used in the further study.

A SNP was assumed to be significant if the Bayes factor (BF; Kass and Raftery, 1995) was ≥ 3.1 , which is termed a 'substantial' effect. The BF was calculated for each SNP by

$$BF = \frac{Pr(H_1|y)}{1 - Pr(H_1|y)} \div \frac{Pr(H_1)}{1 - Pr(H_1)}$$

where H_1 is the hypothesis that the marker is linked to a QTL, $Pr(H_1|y)$ is the posterior probability of a QTL being present and $Pr(H_1)$ is the prior probability of a QTL being present, $(1 - Pr(H_1|y))$ and $(1 - Pr(H_1))$ represent the posterior and prior probability for the alternative hypothesis, respectively. As many SNPs are closely linked and different sets of SNPs might give an equivalent good fit to the data in different chains, the sum of the posterior and prior QTL probabilities of a window of 10 SNPs (a SNP plus its closest neighbours) was also used. Using these summed probabilities might reveal regions with QTL that would be missed otherwise.

The genetic variance of trait i was calculated for each SNP k separately:

$$\sigma'_{ik}{}^2 = 2p_kq_k \times a_{ik}^2$$

where a_{ik} is the allele substitution effect of SNP k on trait i , p_k is the frequency of the minor allele of SNP k and q_k is the frequency of the major allele of SNP k , both obtained from the genotypes. The sum of the variances of all SNPs for trait i was taken as the total genetic variance explained by the SNPs for trait i .

Results

In the entire dataset, 1629 lactations remained (Table 2) after the selection criteria of at least 10 records up to week 15. For BCS mainly records from the UK Langhill herd complied with this criteria, as well as DMI records of the Netherlands and the United Kingdom. Heritability estimates of the four traits were between 0.37 and 0.58. Genetic correlations were of the same sign as their respective phenotypic correlation, but generally stronger (Table 2).

The correlations between r across the genome were 0.95 and 0.97 between seven of the eight cow populations, and between 0.97 and 0.99 with the group of sires. The Langhill control line was somewhat different and r had a correlation of 0.92 with the other cow populations and of 0.95 with the group of sires. The decay in r^2 was similar between the lines (Figure 1), and the combined $N_{e,c}$ parameter was estimated to be 0.107, 0.100, 0.100, 0.107, 0.107, 0.105, 0.100 and 0.105 for Langhill selection and control line (UKC and UKS), NBZ herd, SLU herd, Teagasc herd, the selected and control GEN herd and the group sires, respectively. These $N_{e,c}$ values gave equivalent decay curves for each group, and indicated that N_e of the different subpopulations ranged from 12 462 to 13 344 at average marker distance of 0.08 cMorgans (623 generations ago).

Table 2 Number of cows (n) and estimated heritability, with its standard error (s.e.) per trait, and the genetic (below diagonal; standard errors in parenthesis) and phenotypic (above diagonal; standard errors in parenthesis) correlations among the traits

Trait	n	Heritability	s.e.	Correlations			
				FPCM	DMI	BCS	LW
FPCM	1629	0.37	0.05		0.51 (0.03)	-0.10 (0.04)	0.23 (0.03)
DMI	970	0.53	0.08	0.41 (0.10)		0.17 (0.05)	0.46 (0.02)
BCS	564	0.58	0.09	-0.09 (0.14)	0.39 (0.13)		0.50 (0.03)
LW	1416	0.47	0.07	0.39 (0.11)	0.68 (0.08)	0.52 (0.10)	

FPCM = fat and protein corrected milk; LW = live-weight; BCS = body condition score; DMI = dry matter intake.

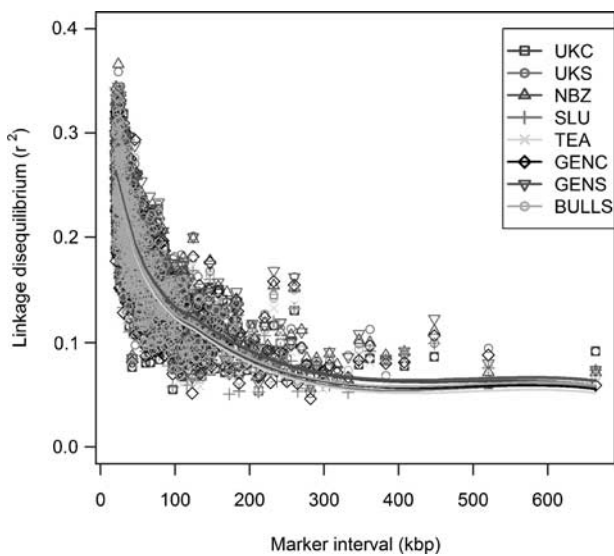


Figure 1 Linkage disequilibrium (LD; r^2) as a function of interval between neighbouring markers for nine different groups: Langhill control and selection line (UKC and UKS), NBZ herd in the Netherlands, SLU herd in Sweden, Teagasc herd in Ireland (TEA), the selected and control herd in the Netherlands (GENS and GENC) and a group of 334 sires of these animals (BULLS).

Across all four traits investigated, 872 SNPs of 37 590 SNPs had a BF >3.1 for at least one trait (Figure 2), whereas 266, 178, 206 and 254 SNPs had a BF >3.1 for LW, BCS, DMI and FPCM, respectively. The number of SNPs with a BF >10 was 36, 18, 19, 22 for LW (Table 3), BCS (Table 4), DMI (Table 5) and FPCM (Table 6), respectively. Using the probabilities across a window of 10 SNPs, in total 300 regions remained significant and 113, 81, 57 and 49 SNPs had a BF above 3.1 for LW, BCS, DMI and FPCM, respectively (Figure 3). The variation explained by the SNPs on each chromosome was related to the size of the chromosome and was relatively consistent for each trait (Table 7), with the possible exceptions of BTA4 for BCS, BTA7, BTA13, BTA14, BTA18 for LW and BTA27 for DMI.

For LW the largest individual BF was on BTA18, BTA7 and BTA14 (Table 3), explaining 0.63%, 0.53% and 0.50% of the total genetic variance explained by SNPs, respectively. All 36 SNPs with a BF >10 explained 2.5% of the genetic variation. Furthermore, four SNPs at BTA13 each explained 0.02% or more of the genetic variance in LW and several other SNPs

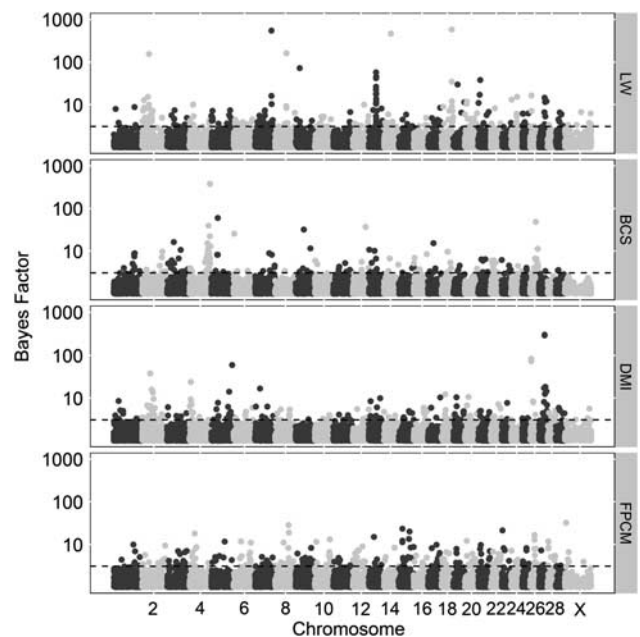


Figure 2 Bayes factor (BF) corresponding to the posterior quantitative trait loci probabilities for each individual single nucleotide polymorphism, from top to bottom, for live-weight (LW), body condition score (BCS), dry matter intake (DMI) and fat and protein corrected milk yield (FPCM) for each of the autosomes from left (BTA1) to right (BTA29) and the X-chromosome at the far right. Dashed lines indicate BF 3.1.

in close proximity to BTA13 were also associated with LW (Figure 4).

A SNP on BTA4 explained 0.68% of the genetic variance in BCS and all 18 SNPs with a BF >10 explained, in total, 1.2% of the genetic variation in BCS (Table 4). For DMI, one SNP on BTA27 explained 0.4% of the genetic variance (Table 5) with several SNPs on predominantly BTA2, BTA26 and BTA27 also explaining genetic variation in DMI. The 19 SNPs with a BF >10 explained 0.8% of the genetic variation.

Of the 872 SNPs associated (i.e. BF >3.1) with at least one of the phenotypes investigated, 31 were associated with two traits and 1 was associated with three traits. This SNP was ARS-BFGL-NGS-71055 on chromosome 27 (34904207 bp) affecting LW, DMI and BCS (Figure 6).

On BTA2, one SNP explained 0.17% of the genetic variance in LW (BF of 153.6) and 0.01% of the genetic variance in DMI (BF of 6.0). Two SNPs in the middle of the putative

Table 3 SNPs that explained most genetic variation for LW with a BF >10 with position on the genome, the posterior probability that the SNP effect is sampled from the distribution with a large effect, the significance (BF) and the variance explained by the single SNP

Chromosome	SNP name	rs	UMD3 position	Posterior probability	BF	SNP variance (BCS units ²)	Total variance (%)
2	BTB-00862061	42025600	8933349	0.004	12.7	0.06	0.01
2	ARS-BFGL-NGS-38895	110915377	31793643	0.005	15.4	0.07	0.01
2	ARS-BFGL-NGS-114956	110791598	39199347	0.044	153.6	0.96	0.17
7	Hapmap46473-BTA-21133	41625106	92293717	0.141	546.8	2.96	0.53
7	ARS-BFGL-NGS-5139	110804446	92474466	0.005	16.1	0.04	0.01
7	ARS-BFGL-NGS-18900	110059753	93218452	0.003	10.3	0.04	0.01
8	BTA-81701-no-rs	41589869	64266000	0.046	161.6	0.97	0.17
9	ARS-BFGL-NGS-104704	110042295	22843876	0.021	71.9	0.34	0.06
13	ARS-BFGL-NGS-95823	109596793	40722564	0.003	10.5	0.04	0.01
13	ARS-BFGL-NGS-41919	109564214	40777908	0.017	57.4	0.24	0.04
13	ARS-BFGL-NGS-116093	109695286	40974987	0.012	42	0.18	0.03
13	ARS-BFGL-NGS-113658	108948219	41015075	0.007	22.5	0.09	0.02
13	BTA-32481-no-rs	41629039	41111195	0.008	26.3	0.11	0.02
13	ARS-BFGL-NGS-60607	109554041	41164722	0.005	17.7	0.07	0.01
13	ARS-BFGL-NGS-101382	109542102	41366870	0.013	45.6	0.22	0.04
13	ARS-BFGL-NGS-16279	110689635	41414256	0.005	18.3	0.08	0.01
13	BTA-26412-no-rs	109799865	41506674	0.003	10.7	0.04	0.01
13	ARS-BFGL-NGS-102025	110274438	41529941	0.004	11.7	0.05	0.01
13	Hapmap61089-rs29014590	29014590	42070556	0.004	13.7	0.05	0.01
14	ARS-BFGL-BAC-21948	109179401	40512939	0.123	465.8	2.79	0.50
18	ARS-BFGL-NGS-98028	41636749	57174711	0.149	581.4	3.54	0.63
18	ARS-BFGL-NGS-109285	109478645	57589121	0.01	34.6	0.14	0.03
18	Hapmap40906-BTA-121147	41664920	58551307	0.004	12.1	0.06	0.01
19	UA-IFASA-6091	41584914	24521970	0.009	29.7	0.14	0.02
19	UA-IFASA-8578	41580816	62760617	0.003	11.4	0.05	0.01
20	ARS-BFGL-NGS-100777	41935177	9965585	0.003	10.6	0.07	0.01
20	ARS-BFGL-NGS-71611	110815453	11035444	0.004	11.9	0.06	0.01
21	ARS-BFGL-NGS-31507	110426938	2592694	0.003	11	0.05	0.01
21	ARS-BFGL-NGS-100241	110440906	8955497	0.005	17.1	0.1	0.02
21	Hapmap55337-rs29009998	29009998	11981034	0.011	37.8	0.18	0.03
24	ARS-BFGL-NGS-63149	42039872	3721378	0.004	13.1	0.05	0.01
24	Hapmap57214-rs29016076	29016076	34928812	0.005	15.4	0.09	0.02
26	ARS-USMARC-Parent-DQ990834	29013727	8221270	0.005	16.4	0.07	0.01
27	Hapmap40631-BTA-103396	41615000	31461111	0.004	14.5	0.06	0.01
27	ARS-BFGL-NGS-29650	42634874	34326743	0.003	11.5	0.05	0.01
27	Hapmap50424-BTA-63130	110518457	41726707	0.004	12.1	0.06	0.01

SNP = single nucleotide polymorphism; LW = live-weight; BF = Bayes factor; BCS = body condition score.

QTL region on BTA13 for LW (Figure 4) had a BF >3.1 for BCS and DMI as well. Similarly, SNPs associated with LW were in close proximity to the putative QTL region for BCS on BTA26 (Figure 5), and on BTA27 several SNPs were associated (i.e. BF >3.1) with FPCM, DMI, LW and BCS (Figure 6).

Discussion

Genetic variation

The heritability estimates of 0.37 to 0.58 are similar to the estimates previously reported for these traits (Koenen and Veerkamp, 1998; Berry *et al.*, 2003). The increased DMI for heavier animals and animals that yield more milk is expected and agrees with previous studies as does the phenotypic and genetic correlations of 0.50 to 0.52 between BCS and LW (Berry *et al.*, 2002). The general pattern that the genetic

variance explained by SNPs on each chromosome decreases with the decreasing size of the chromosome is consistent with a polygenic model. Exceptions to this general rule that genetic variance explained by SNPs is related to the size of the chromosome are BTA7, BTA13, BTA14 and BTA18 for LW, BTA4 for BCS and BTA27 for DMI. These chromosomes explain more variance than expected in the case that variance were uniformly distributed across the genome.

The chromosomes that explain more genetic variance than expected relative to their size are also the chromosomes with most significant SNPs for these traits. Nevertheless, SNPs explained only a small proportion of the overall variance. With a maximum of 0.7% of the variance explained by a single SNP, it is expected that thousands of QTL are required to explain the full variance and the effect is smaller than is expected given the power of the experiment.

Table 4 SNPs that explained most genetic variation for BCS with a BF >10 with position on the genome, the posterior probability that the SNP effect is sampled from the distribution with a large effect, the significance (BF) and the variance explained by the single SNP

Chromosome	SNP name	rs	UMD3 position	Posterior probability	BF	SNP variance (BCS units ²)	Total variance (%)
3	BTA-91560-no-rs	41661577	38507659	0.005	16.3	0.0000064	0.02
3	BTA-68406-no-rs	41608825	77666001	0.003	10.7	0.0000045	0.02
4	ARS-BFGL-NGS-31561	109395087	102433510	0.005	15.4	0.0000044	0.02
4	ARS-BFGL-NGS-72134	110800831	106253833	0.003	11.3	0.0000036	0.01
4	BTA-90241-no-rs	41612738	107985246	0.012	39.4	0.0000119	0.04
4	Hapmap28815-BTA-155553	81169737	116390964	0.007	22.2	0.0000065	0.02
4	Hapmap60162-rs29015028	29015028	116485311	0.102	377.2	0.0001796	0.68
4	ARS-BFGL-NGS-33132	41669146	119617605	0.004	13.1	0.0000037	0.01
5	BTB-00225371	43435407	36578127	0.018	59.9	0.0000228	0.09
6	BTB-01280976	42404150	4193024	0.008	25.7	0.0000075	0.03
9	BTA-83597-no-rs	41658682	46632366	0.009	31.8	0.000009	0.03
12	ARS-BFGL-NGS-28261	110940900	72311316	0.011	36.6	0.0000121	0.05
13	ARS-BFGL-BAC-11281	109707704	2217706	0.003	10.8	0.0000028	0.01
13	ARS-BFGL-NGS-69389	109983109	32831454	0.003	10.1	0.0000034	0.01
17	BTB-00676214	41840165	30540080	0.005	15.4	0.0000049	0.02
26	BTB-02036417	43145430	32716808	0.014	48.2	0.0000185	0.07
26	Hapmap52062-rs29027270	29027270	43479080	0.003	11.3	0.0000034	0.01

SNP = single nucleotide polymorphism; BF = Bayes factor; BCS = body condition score.

Table 5 SNPs that explained most genetic variation for DMI with a BF >10 with position on the genome, the posterior probability that the SNP effect is sampled from the distribution with a large effect, the significance (BF) and the variance explained by the single SNP

Chromosome	SNP name	rs	UMD3 position	Posterior probability	BF	SNP variance (BCS units ²)	Total variance (%)
2	Hapmap59844-rs29025329	29025329	44947955	0.011	37.9	0.00035	0.05
2	BTB-01559051	42674303	53431931	0.005	15.9	0.00013	0.02
2	ARS-BFGL-NGS-4659	109704643	60143778	0.004	13.6	0.00016	0.02
3	BTA-68406-no-rs	41608825	77666001	0.003	10.7	0.0000045	0.02
4	ARS-BFGL-NGS-114611	110661405	8421365	0.007	23.9	0.00015	0.02
5	BTA-116856-no-rs	41618337	100826813	0.004	14.2	0.00009	0.01
5	ARS-BFGL-NGS-14632	110117542	118501191	0.018	59.9	0.00048	0.06
7	BTB-01074080	42232105	27973088	0.005	16.7	0.0001	0.01
13	ARS-BFGL-BAC-11281	109707704	2217706	0.003	10.8	0.0000028	0.01
13	ARS-BFGL-NGS-69389	109983109	32831454	0.003	10.1	0.0000034	0.01
18	BTA-42769-no-rs	41579997	20765346	0.004	12.2	0.00009	0.01
26	BTB-01078268	42233405	7846224	0.024	83.3	0.00068	0.09
26	BTB-01078331	42234268	7900988	0.021	72.5	0.00063	0.08
26	Hapmap52062-rs29027270	29027270	43479080	0.003	11.3	0.0000034	0.01
27	Hapmap43421-BTA-97718	41666656	28148660	0.005	17.2	0.00014	0.02
27	Hapmap40631-BTA-103396	41615000	31461111	0.081	295.4	0.00303	0.40
27	ARS-BFGL-NGS-112047	43727261	33442942	0.004	12.6	0.00012	0.02
27	ARS-BFGL-NGS-108861	110320533	34758932	0.006	18.4	0.00014	0.02
27	Hapmap50424-BTA-63130	110518457	41726707	0.004	14.4	0.00012	0.02

SNP = single nucleotide polymorphism; DMI = dry matter intake; BF = Bayes factor; BCS = body condition score.

An explanation for the small proportion of variance explained by each SNP might be that all SNPs were fitted simultaneously in the model and in comparison with single SNP analysis, this might have reduced the total variance explained by each SNP. There might not be enough discriminant power in the data to separate the SNPs close together, as several SNPs are expected to be in LD with each

other, and the effects of the same QTL will be distributed across several SNPs. In addition, the average SNP effects across five chains were taken to avoid a single SNP being highly significant in one chain, but the effect being attributed to a neighbouring SNP in subsequent chain. The effect of several SNPs in close proximity of each other explaining each a little of the genetic variance is, for example, illustrated by

Table 6 SNPs that explained most genetic variation for FPCM with a BF >10 with position on the genome, the posterior probability that the SNP effect is sampled from the distribution with a large effect, the significance (BF) and the variance explained by the single SNP

Chromosome	SNP name	rs	UMD3 position	Posterior probability	BF	SNP variance (BCS units ²)	Total variance (%)
0	BTA-69349-no-rs	43741236	0	0.004	13.2	0	0.01
4	Hapmap38265-BTA-96973	41592066	30526269	0.005	18	0.001	0.02
5	Hapmap46673-BTA-74066	41566931	75205122	0.003	11.7	0	0.01
6	BTA-75902-no-rs	43455987	42155077	0.004	11.7	0	0.01
6	Hapmap36567-SCAFFOLD30438_8760	29016199	97937238	0.003	11	0	0.01
8	ARS-BFGL-NGS-118200	110589243	49203188	0.003	11.1	0	0.01
8	BTA-81945-no-rs	41658302	77252882	0.009	28.7	0.001	0.02
8	BTB-01139515	42296470	78701950	0.006	18.9	0.001	0.01
10	ARS-BFGL-NGS-5922	110672262	9789845	0.003	11.5	0	0.01
10	BTB-00441734	43651067	85944517	0.004	13.2	0	0.01
13	ARS-BFGL-NGS-113236	109655563	28989009	0.004	15	0.001	0.01
15	BTB-00582624	41745388	21138866	0.003	11.3	0	0.01
15	BTB-02053380	43160689	21162454	0.004	12.3	0	0.01
15	BTB-00582817	41746981	21401836	0.007	23.5	0.001	0.02
15	ARS-BFGL-BAC-6489	110748087	58411725	0.004	13.4	0.001	0.01
15	BTB-01059544	42218318	61098317	0.006	20	0.001	0.02
20	Hapmap59100-rs29024884	29024884	11770435	0.004	12.1	0	0.01
23	ARS-BFGL-NGS-106470	110809507	5047747	0.006	21.3	0.001	0.02
26	Hapmap53060-rs29020888	29020888	25032529	0.005	16.7	0	0.01
26	ARS-BFGL-NGS-25560	42097667	25407197	0.004	13	0	0.01
28	BTA-100905-no-rs	41612720	4866330	0.004	11.7	0	0.01
30	Hapmap41447-BTA-118863	41573451	7268125	0.01	32.3	0	0.01

SNP = single nucleotide polymorphism; FPCM = fat and protein corrected milk; BF = Bayes factor; BCS = body condition score.

Table 7 Percentage of total SNP variance[†] of LW, BCS, DMI and FPCM explained by SNPs on each chromosome

Chromosome	LW (%)	BCS (%)	DMI (%)	FPCM (%)	Chromosome	LW (%)	BCS (%)	DMI (%)	FPCM (%)
1	5.8	6.0	6.0	6.1	16	3.0	3.0	3.1	3.0
2	5.3	5.1	5.3	5.2	17	2.9	2.9	2.9	2.9
3	4.6	4.8	4.7	4.7	18	3.1	2.5	2.5	2.5
4	4.6	5.6	4.7	4.7	19	2.6	2.6	2.6	2.6
5	4.0	4.1	4.1	4.0	20	2.8	2.8	2.8	2.8
6	4.6	4.7	4.7	4.8	21	2.7	2.7	2.7	2.7
7	4.7	4.1	4.1	4.2	22	2.4	2.5	2.5	2.5
8	4.3	4.1	4.2	4.3	23	2.0	2.1	2.1	2.1
9	3.8	3.8	3.8	3.9	24	2.3	2.3	2.3	2.3
10	4.0	4.1	4.0	4.1	25	2.0	2.0	2.0	2.0
11	4.1	4.2	4.2	4.2	26	1.9	2.1	2.1	2.0
12	3.1	3.2	3.1	3.2	27	1.8	1.8	2.3	1.8
13	3.7	3.5	3.5	3.5	28	1.7	1.8	1.8	1.8
14	3.8	3.4	3.4	3.4	29	2.1	2.1	2.1	2.1
15	3.1	3.1	3.1	3.3	X	1.9	1.9	1.9	1.9

SNP = single nucleotide polymorphism; LW = live-weight; BCS = body condition score; DMI = dry matter intake; FPCM = fat and protein corrected milk.

[†]1.4% of the genetic variance was explained by SNPs not mapped to chromosomes yet.

the SNPs associated with LW on BTA13 (Figures 2 and 4). At the same time on BTA14 one SNP associated with LW was found, whereas no other neighbouring SNPs were found. However, this SNP explained only 0.5% of the variance, suggesting that the most likely scenario is that many mutations, all with a relatively small effect, explain the total genetic variance for LW, DMI and BCS.

Functional processes relating to SNPs associated with DMI, LW and BCS

In total, 728 genes and 500 unique genes were found within 500 kbp flanking the SNPs associated with LW, DMI or BCS using BioMart (Haider *et al.*, 2009). Fourteen of these genes were present in proximity of the SNPs associated with both LW and DMI, and 27 in proximity of the SNPs associated with

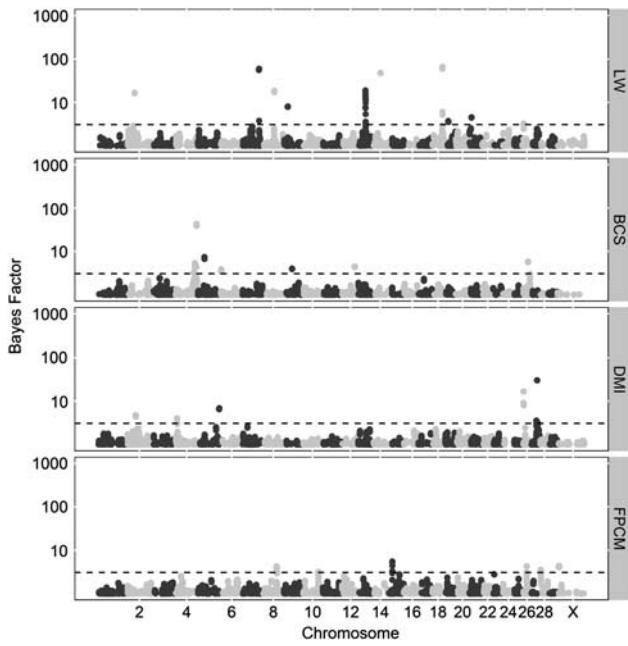


Figure 3 Posterior Bayes factor (BF) corresponding to the posterior quantitative trait loci probabilities for a window of 10 neighbouring single nucleotide polymorphisms, from top to bottom, for live-weight (LW), body condition score (BCS), dry matter intake (DMI) and fat and protein corrected milk yield (FPCM) for each of the autosomes from left (BTA1) to right (BTA29) and the X-chromosome at the far right. Dashed lines indicate BF 3.1.

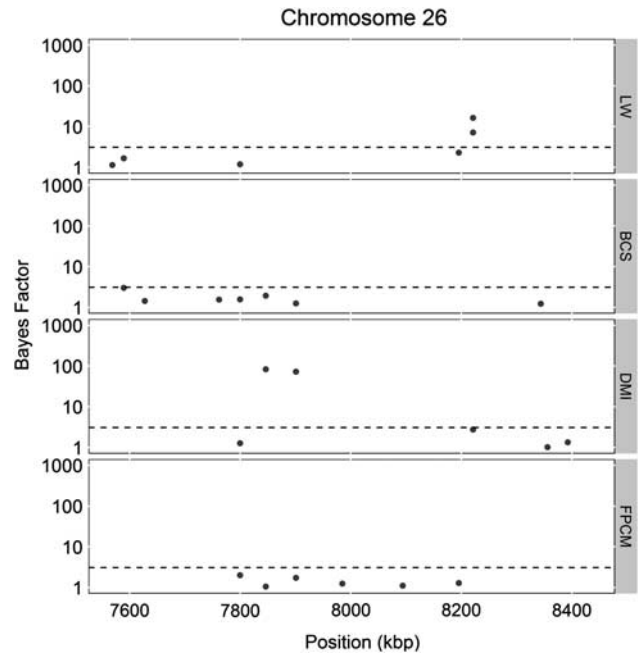


Figure 5 Bayes factor (BF) corresponding to the posterior quantitative trait loci probabilities for single nucleotide polymorphism with $BF > 1$ on a region of chromosome 26 (BTA 26) for live-weight (LW), body condition score (BCS), dry matter intake (DMI) and fat and protein corrected milk yield (FPCM). Dashed lines indicate BF 3.1.

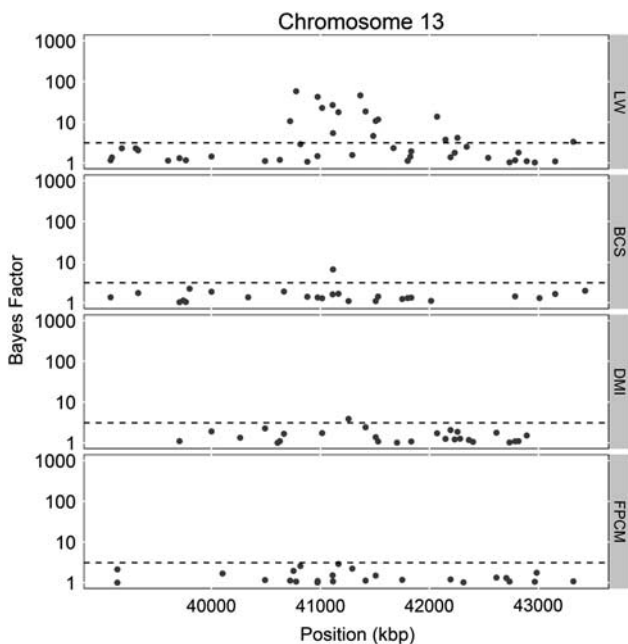


Figure 4 Bayes factor (BF) corresponding to the posterior quantitative trait loci probabilities for single nucleotide polymorphism with $BF > 1$ on a region of chromosome 13 (BTA13) for live-weight (LW), body condition score (BCS), dry matter intake (DMI) and fat and protein corrected milk yield (FPCM). Dashed lines indicate BF 3.1.

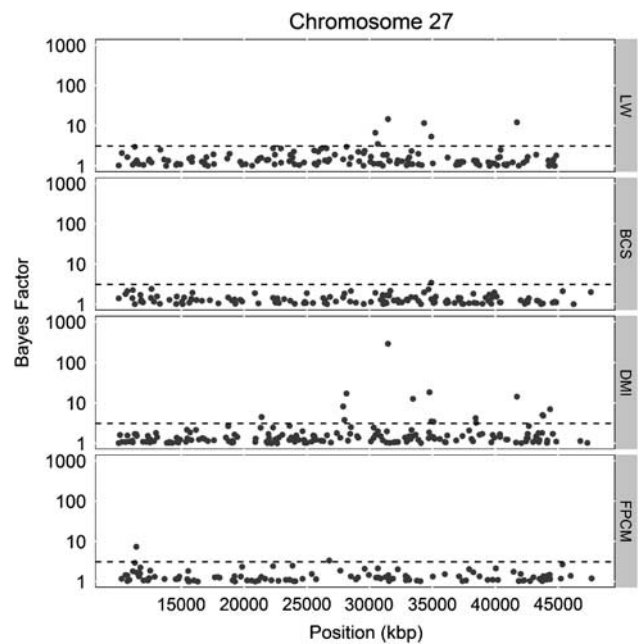


Figure 6 Bayes factor (BF) corresponding to the posterior quantitative trait loci probabilities for single nucleotide polymorphism with $BF > 1$ on a region of chromosome 27 (BTA 27) for live-weight (LW), body condition score (BCS), dry matter intake (DMI), and fat and protein corrected milk yield (FPCM). Dashed lines indicate BF 3.1.

both BCS and DMI. Clustering the 500 genes on gene function (Huang *et al.*, 2009a) showed that three gene groups were overrepresented. The first gene group (enrichment score 5.66) contained 16 genes involved with kallikrein,

peptidase and trypsin. The second gene group contained five genes involved with cystan (enrichment score 1.86) and, interestingly, the third group contained 32 olfactory, taste receptor and pheromone receptor genes. The latter suggests

that smell and taste genes play an important role in the feed intake complex. When clustered on functional process, peptidase activity, epidermal growth factor (EGF)-like sensory smell, olfactory and catabolic processes were enriched.

Clustering was also performed for each of the genes associated to each trait separately. When the 227 genes close to the SNPs for BCS were clustered, the group of 16 olfactory genes remained enriched (score 2.40), but one cluster also contained the trypsin-related genes. Clustering the 111 genes in proximity of the SNPs associated with DMI gave one enriched group with zinc finger and ADAM metallopeptidase genes. Functionally, clustering gave a group with EGF-like processes, and a cluster with leptin-type processes, zinc- and iron-binding processes and peptidase. For the trait LW, 11 genes out of 257 were clustered for the kallikrein-related peptidase (enrichment 4.66) and cystatin (enrichment 1.65). Nevertheless, most of these processes have very general functions, and it is difficult to anticipate any direct target for application apart from perhaps the olfactory genes and sensory smell process that might indicate the importance of genetic variation in the genes affecting taste and smell for the feed intake complex of dairy cows.

Candidate genes affecting DMI, LW and BCS

Several candidate genes have been investigated in cattle. For example, the leptin gene on BTA4 (Van der Lende *et al.*, 2005; Banos *et al.*, 2008), LEPR on BTA3 (Liefers *et al.*, 2005; Banos *et al.*, 2008), SCD on BTA26 (Macciotta *et al.*, 2008), PGF on BTA10 (Seidenspinner *et al.*, 2011), SPP1 on BTA6 (Sheehy *et al.*, 2009), GH on BTA19 (Mullen *et al.*, 2010) and GHR on BTA20 (Banos *et al.*, 2008; Waters *et al.*, 2011). For all these candidate genes we looked at the groups of SNPs positioned surrounding these genes. However, no strong associations ($BF > 10$) were found for the SNPs in the vicinity of these genes. This does not exclude that these genes play a role in the genetic variation for these traits, but the Bayesian variable selection method used here did not select any of these surrounding segregating SNPs. This might be because the effect is explained by many SNPs in LD with the important SNPs within these genes, or the SNPs used here are not in strong LD with functional SNPs. Therefore, for each of the traits new candidate genes were investigated by focussing first on the 22 SNPs that were identified with $BF > 3.1$ and that were located in known genes (<http://www.ensembl.org>, Btau_4.0; April 2010) and second by identifying the genes around the SNPs with an extreme effect ($BF > 50$).

For LW, seven SNPs had a $BF > 50$, of which three SNPs had a very strong association ($BF > 460$) and explained close to 0.5% of the genetic variation. The first SNP on BTA18 (ARS-BFGL-NGS-98028) is located in the *syt3* gene. This gene is from a family of genes that are known to affect the exocytosis of insulin that in turn influences plasma insulin levels (Gauthier and Wollheim, 2008). In this gene, a putative QTL for LW was identified before in a beef F2 population (MacNeil and Grosz, 2002). Interestingly, ARS-BFGL-NGS-38895 on BTA2 was also associated with LW, and within 5 kbp of this SNP the gene *GRB14* was found. This gene encodes a growth factor receptor-binding protein that interacts with insulin

receptors and IGF receptors and may play a role in signalling pathways that regulate growth and metabolism. The second SNP with a strong association with LW was located on BTA7 (Hapmap46473-BTA-21133) and is located in the gene *CETN3*. The third SNP (ARS-BFGL-BAC-21948) with a very strong association ($BF > 460$) was not located in a gene. Of the four other SNPs associated to LW with $BF > 50$, only BTA-81701-no-rs was located in *anks6* on BTA8.

For BCS, three SNPs with a large effect were located in known genes: BTB-00225371 on BTA5 ($BF > 59$) in *TMEM117* and, the two SNPs on BTA4 (ARS-BFGL-NGS-33132 and ARS-BFGL-NGS-31561) were mapped to genes *Ptprn2* and *CREB3L2*.

For DMI, three SNPs were mapped on genes: BTB-01074080 was mapped on *MEGF10*, ARS-BFGL-NGS-108861 was mapped on *IDO2* and Hapmap59844-rs29025329 was mapped on *N-myc* (and *STAT*) interactor. *IDO2* might be an interesting candidate gene. The gene *IDO2* plays an important role in tryptophan metabolism. Tryptophan is an essential amino acid that cannot be produced in the body. For that reason, tryptophan is well investigated and linked to feed intake in humans (Wolfe *et al.*, 1997), cattle (Choung and Chamberlain, 1992), mice (Coskun *et al.*, 2006) and pigs (Montgomery *et al.*, 1980). As discussed by Koopmans *et al.* (2006), tryptophan serves as the immediate precursor to serotonergic activity in the brain, and has been implicated in the regulation of many behavioural and physiological processes such as mood, aggression, susceptibility to stress, sleep patterns, but also the regulation of feed intake. Hence, *IDO2* on BTA27 might be an ideal candidate gene to affect DMI.

Another set of candidate genes for DMI might be those involved in EGFs and transforming growth factors. The *MEGF10* gene, in which a SNP that was associated with DMI was identified, clustered on function with three other genes that were in proximity to the significant SNPs (enrichment score 2.04). The three genes were *ADAM2* on BTA27 (within 500 kbp of ARS-BFGL-NGS-108861 that was associated with DMI and within 5 kbp of ARS-BFGL-NGS-29650 that was associated with LW), *ADAM3A* on BTA27 (within 50 kbp of ARS-BFGL-NGS-29650 that was associated with LW) and *NRG1* on BTA27 (close to Hapmap43421-BTA-97718 that was associated with DMI). EGFs are involved in cell recognition and division (Campbell *et al.*, 1990), and functional analysis suggest that these set of genes played a role in lean and obese mice (Fuller *et al.*, 2007).

Overall a few new candidate genes have been identified for the feed intake complex. These candidate genes are linked to insulin, EGF and tryptophan.

Conclusion

Genome-wide association study of data collected across five experimental herds identified 872 SNPs associated with DMI, BCS, LW and FPCM. The genetic variance in each trait explained by the SNPs on a chromosome was generally proportional to the size of the chromosome. Next to genes involved in some general processes, the olfactory genes and

genes involved with sensory smell processes appeared to be present in the region of 500 kbp of the significant SNP(s). Potential candidate genes, based on function of the gene and location of a significant SNP in a gene, were linked to insulin, EGF and tryptophan.

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