Effect of Quantitative Trait Loci for Milk Protein Percentage on Milk Protein Yield and Milk Yield in Israeli Holstein Dairy Cattle

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ABSTRACT

Although numerous quantitative trait loci (QTL) mapping studies involving milk protein percent (PP), milk yield (MY), and protein yield (PY) have been carried out, there has not been any systematic evaluation of the effects of individual QTL on these 3 interrelated traits. Consequently, the aim of the present study was to investigate the effects on MY and PY of QTL for PP previously mapped in various laboratories. The study, based on selective DNA pooling of milk samples, included 10 Israeli Holstein artificial insemination bulls, each the sire of 1,800 or more milk-recorded daughters. For each sire-trait combination across the 10 sires, milk samples of the highest and lowest daughters with respect to estimated breeding values for PP, PY, and MY were collected for pooling. A total of 134 dinucleotide microsatellites distributed over 25 bovine autosomes were used. An empirical standard error for marker-QTL linkage testing was calculated based on the variation among split samples within the same tail. Threshold comparison-wise error rate *P*-values were set to control proportion of false positives at *P* = 0.10 level for declaring significant effects at the marker-trait level. Estimates of the number of true null hypotheses for each trait were obtained from the histogram of marker comparison-wise error rate *P*-values. Based on these estimates, effective power of the experiment at the marker-trait level was estimated as 0.75, 0.41, and 0.73 for PP, PY, and MY. The proportion of heterozygosity at the QTL was estimated as 0.46, 0.39, and 0.40, respectively. After correcting for incomplete power and proportion of false positives, it was estimated that 38.7 and 37.5% of the markers affecting PP and MY, respectively, also affected PY. Of the markers affecting PY, 68.9 and 76.5%, respectively, also affected PP and MY. Apparently, none of the significant markers affected PY exclusively, and only 6.5 and 16.0%, respectively, affected PP or MY exclusively. Thus, almost all significant markers,

and by inference almost all QTL, had effects on at least 2 of the 3 traits.

Key words: quantitative trait loci mapping, selective DNA, proportion of false positive

INTRODUCTION

A large number of studies have consistently documented a very high positive genetic correlation between milk yield (MY) and protein yield (PY), a moderate negative genetic correlation between MY and protein percentage (PP), and a very low, although generally positive, genetic correlation between PP and PY (Sölkner, 1989; Chauhan and Hayes, 1991; Welper and Freeman, 1992). For marker-assisted selection, it is important to know whether these relationships hold at the level of the individual QTL, or if there are QTL that have exclusive effects on PP, MY, or PY. Estimates of effects of β -CN A1 and A2 alleles and for the DGAT K232A allele on PP, MY, and PY were reported previously and are summarized in Table 1. Overall, directions of effects of the various alleles are consistent across populations and are consistent with the genetic correlations among the traits, namely specific alleles affect MY and PY in the same direction and PP in the opposite direction. On the other hand, estimated effects of specific alleles on the individual traits change in different populations. This may be due to sampling, to interaction of the allele with genetic background of the population, or to differences in the DNA sequence of the allele in different populations. On average, standardized effects of β -CN and DGAT alleles on PP were the same, but DGAT alleles appeared to have a greater effect on MY than β -CN alleles; the opposite was true for the effects of DGAT and β -CN alleles on PY. Thus, effects of individual alleles on the 3 traits may differ among genes. Viitala et al. (2003) used combined analysis of multiple chromosomes to map QTL affecting PP, MY, and PY in Finnish Ayrshire dairy cattle, at relatively high power. Assuming that QTL affecting PP, MY, or PY that map to within 20 cM of one another represent multiple effects of the same QTL, results by Viitala et al. (2003) can be interpreted as having identified 12 QTL affecting MY, of which 5 (45%) also affected

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Table 1. Absolute and standardized effects of β -CN/A1A1, β -CN/A2A2, and DGAT/K232A alleles on protein percentage (PP), protein yield in kilograms (PY), and milk yield in kilograms (MY) in various published studies¹

Gene/allele	Study	Population	PP	PY	MY
β -CN/A1A1	Freyer et al. (1999)	SMR/HF ¹	0.014	-2.15	-83
		DSR ¹	0.007	-1.31	-93
β -CN/A2A2		SMR/HF	-0.018	3.68	124
		DSR	-0.058	1.01	51
DGAT/K232A	Grisart et al. (2001)		0.040	-2.82	-158
	Kühn et al. (2004)		0.008	-0.98	-48
	Spelman et al. (2002)	Holstein	ND	-2.45	-134
	Thaller et al. (2003)	Holstein ²	0.051	-0.67	-108
		Fleckvieh ²	-0.013	-0.79	-146
	Weller et al. (2003)	Holstein	0.057	-3.1	-306
Mean β -CN			-0.024	2.04	87.5
In σ_A units			-0.16	0.11	0.14
Mean DGAT			0.024	-1.37	-140.7
In σ_A units			0.16	-0.07	-0.23

¹HF = Holstein-Friesian; SMR = HF $\times$ Jersey; SMR/HF = SMR $\times$ HF; DSR = German Black and White.²Mean of first, second, and third lactations.

PY, and 6 QTL affecting PP, of which only 1 affected PY (Table 2). Thus, the Viitala et al. (2003) study indicates that a majority of QTL affecting MY or PP did not affect PY and that effects of individual sire haplotypes on MY and PP were in opposite directions. On the basis of these results, Viitala et al. (2003) proposed that many QTL affect the volume of the liquid component of milk through primary effects on concentration of osmotic molecules (such as lactose and some of the Ca and P components). Such QTL would affect MY and PP in opposite directions and hence would be essentially neutral with respect to PY. Recently, Bagnato et al. (2008) reported a genome-wide scan of Brown Swiss dairy cattle for PP and MY using selective DNA pooling (Darvasi and Soller, 1994; Lipkin et al., 1998; Mosig et al., 2001). They identified a total of 55 QTL regions (QTLR), of which 16 affected MY exclusively and 13

affected PP exclusively. Eight of the 16 QTLR exclusively affecting MY, and 10 of the 13 exclusively affecting PP, also had reported results in 1 or more of the 3 Web cattle QTL databases (Cattle QTLdb, Hu et al., 2007; QTL Map, Khatkar et al., 2004; QTL Viewer, Polineni et al., 2006). Remarkably, all 8 QTLR in the Brown Swiss that exclusively affected MY were also found to exclusively affect MY in the databases. Similarly, all 10 QTL in the Brown Swiss that exclusively affected PP also exclusively affected PP in the databases. Following the Lander and Kruglyak (1995) validation criteria, and considering that the databases are based mainly on data obtained from Holstein populations, the completely independent replicated specificity of effects across different populations can be taken as a validation of these specific effects. Thus, in the study by Bagnato et al. (2008), an appreciable fraction of QTL appear to affect MY or PP primarily or exclusively, with little or no effect on the other trait.

We have previously reported extensive QTL-mapping studies for PP in the Israel Holstein dairy cattle population using selective DNA pooling of milk samples (Lipkin et al., 1998; Mosig et al., 2001). The aim of the present study, also based on selective DNA pooling, was to determine the pleiotropic effects of QTL affecting PP on the 2 closely related traits PY and MY and to develop a method to correct the observed proportion of multiple effects for incomplete power.

MATERIALS AND METHODS

Selective DNA Pooling

Large half-sib or full-sib families are routinely produced in dairy cattle populations as part of routine genetic improvement programs. The size of those fami-

Table 2. Quantitative trait loci location mapping results of Viitala et al. (2003) for protein percentage (PP), protein yield (PY), and milk yield (MY) according to chromosome (*Bos taurus* autosome, BTA) and location (cM)

BTA	cM		MY
	PP	PY	
1			137
2			35
3	1		62
5		77	94
6	66		66
12	10	16	12
14	50		
20	68		82
21			24
23	21		4
25		66	70
27		29	29
29		28	34

Table 3. Data sets used in this study¹

Data set	Sires	Year	Traits	Role	Comment and publication
DS1	4	1996	PP	Mapping	Lipkin et al., 1998 Mosig et al., 2001
DS2	4	1998	PP, MY	Mapping	New daughters of the sires sampled in DS1
DS3	6	1998	PP, PY, MY	Mapping	New sample
DS4	7	1996	PP	SE(D')	Includes the 4 sires of DS1 Lipkin et al., 1998 Mosig et al., 2001
DS5	7	2002	PP	SE(D')	New sample, including 3 sires of DS3
DS6	6	2003	FF, SCC	SE(D')	New sample, including 5 sires of DS5

¹PP = protein percentage; PY = protein yield; MY = milk yield; FF = female fertility; SE(D') = calculation of empirical standard error of D.

lies provide statistical power for QTL mapping within population, which can be accessed by use of a daughter design (Soller and Genizi, 1978; Weller et al., 1990). However, achieving the high power theoretically available with the daughter design requires genotyping very large numbers of daughters against large numbers of markers, resulting in high genotyping costs (Soller, 1994). These costs can be greatly reduced by the use of selective DNA pooling (Darvasi and Soller, 1994; Lipkin et al., 1998; Mosig et al., 2001). In this procedure, determination of linkage between a molecular marker and a QTL is based on the distribution of parental marker alleles among pooled DNA samples of the extreme high and low phenotypic groups of the offspring population. Densitometric estimates of sire allele frequencies in the pools were obtained after correction for shadow bands, as previously described (Lipkin et al., 1998; Mosig et al., 2001).

Population and Samples

The study included 10 half-sib daughter families of Israeli Holstein AI bulls, each the sire of 1,800 or more milk-recorded daughters and 134 markers. This would have required a total of almost 2 million genotyping data points if done by individual genotyping. Thus, the study was made feasible only through selective DNA pooling, which enabled it to be carried out with a total of about 25,000 genotyping data points. Herd book data and milk samples of these daughters were collected at different times, generating a series of data sets (DS), which are summarized in Table 3. In particular, daughters of 4 of the sires, comprising DS1 were previously sampled and mapped for PP in 1996. In 1998, for purposes of the present study, daughters of these 4 sires were independently resampled for PY and MY (DS2), and 6 additional sire families were sampled for all 3 traits (DS3). Based on EBV, lists of the highest and lowest 220 (DS1) or 230 (DS2, DS3) daughters were prepared for each sire-trait-tail combination. Milk sam-

pling was as previously described (Lipkin et al., 1998; Mosig et al., 2001). For each sire-trait-tail combination, 2 subpools (DS1) or a single pool (DS2, DS3) were prepared in completely independent duplicate. Genotyping of individual and pooled milk samples was as described (Lipkin et al., 1998). Densitometric values for the marker and sire-marker tests of DS1 were obtained from the original data of Lipkin et al. (1998) and Mosig et al. (2001) as described in those studies. These were reanalyzed using the empirical standard error obtained in the present study. All other densitometric values were obtained on the basis of data collected in the present study.

In addition to the above samples used for mapping the 3 traits, data from daughter samples DS4, DS5, and DS6 were used to calculate the empirical standard error (see below) but were not otherwise included in the present study.

Microsatellite Markers

A total of 134 dinucleotide microsatellites distributed over 25 bovine autosomes were used to scan chromosomal intervals indicated previously to harbor QTL affecting PP (Lipkin et al., 1998; Mosig et al., 2001). All markers were obtained from the public Web sites (USDA, <http://www.marc.usda.gov/genome/genome.html>; IBRP, <http://www.cgd.csiro.au/cgd1.html>).

Marker-QTL Linkage Tests

The test for linkage between a marker and each of the 3 study traits was carried out at 2 levels: the sire-marker-trait level, which tests for association of marker and trait at the level of the individual sire, and the marker-trait level, which tests for association of marker and trait across all sires.

Sire-Marker-Trait Combinations. For each sire-marker-trait combination for which the sire was heterozygous at the marker, the comparison-wise error rate

(CWER) P_{ijk} -value, for the i th sire- j th marker- k th trait combination was obtained as twice the area of the normal curve from Z_{ijk} to $+\infty$, where

$$Z_{ijk} = D_{ijk}/SE(D'_{ijk}).$$

$D_{ijk} = (DL_{ijk} - DS_{ijk})/2$ is the difference in sire-allele frequencies between the high and low daughter pools of the i th sire with respect to the j th marker and the k th trait, averaged over the long and short sire alleles (Lipkin et al., 1998; Mosig et al., 2001). DL_{ijk} is the difference in allele frequency of the long sire allele between the high and low daughter pools. DS_{ijk} is the difference in allele frequency of the short sire allele between the high and low daughter pools. DL_{ijk} and DS_{ijk} will necessarily have opposite algebraic sign; hence, the minus sign in the expression for D_{ijk} .

Empirical SE(D'). Lipkin et al. (1998) and Mosig et al. (2001) calculated SE(D) on a priori grounds, on the assumption that it was determined by binomial sampling of sire and dam alleles and technical error of densitometric estimation of allele frequency in a pool. For the present study a novel empirical standard error was used, denoted SE(D'), based on a D-value (**Dtail**), obtained between 2 independent subpools at the same tail. Details on calculation of SE(D') are presented in Bagnato et al. (2008). To obtain an estimate of SE(D') for use in the present study, we reanalyzed 3 datasets (DS4, DS5, DS6; Table 3) for which 2 subpools were prepared for each tail. Data set 4 consisted of high and low daughters according to EBV for PP, of each of 7 Israel-Holstein sires. For each sire, the daughters comprising each tail were divided into 2 subpools. The 2 subpools in each tail were not completely independent, because the most extreme daughters within tails were assigned to the external subpools and the remainder to internal subpools (Lipkin et al., 1998; Mosig et al., 2001). Nevertheless, average Dtail was not significantly different from zero (data not shown), allowing the use of this DS to estimate SD(Dtail). Data set 5 consisted of high and low daughters according to EBV for PP of a new set of 7 sires. For each sire, the daughters in each tail were divided at random among 2 independent subpools. Data set 6 consisted of high and low daughters according to EBV for female fertility and a second set of high and low daughters according to EBV for milk SCC for 6 sires, 5 of which were included in DS5 and 1 which was collected independently. Here too, for each sire, the daughters in each tail were divided at random among 2 independent subpools.

Marker-Trait Combinations. The CWER P_{jk} -value, for the j th marker- k th trait combination, was obtained as the area of the χ^2 distribution from χ_{jk}^2 to $+\infty$, where

$$\chi_{jk}^2 \text{ (df} = s_{i(jk)}) = \sum_{i(jk)} Z_{i(jk)}^2.$$

The parentheses in the subscript of $\sum_{i(jk)}$ and $Z_{i(jk)}^2$ indicate that the summation is over sires heterozygous at the j th marker, within the j th marker- k th trait combination, and $s_{i(jk)}$ is the number of heterozygous sires for the jk th marker-trait combination (Weller et al., 1990; Lipkin et al., 1998; Mosig et al., 2001).

Proportion of True and Falsified Null Hypotheses Out of All Tests. The total number of tests (N) represents a mixture comprised of n_1 tests for which the null hypothesis is false (i.e., marker-QTL linkage is present) and n_2 tests for which the null hypothesis is true (i.e., marker-QTL linkage is not present). Thus, the observed distribution of CWER P -values is a mixture of P -values representing these 2 classes of tests. On this basis, given the observed distribution of CWER P -values for the individual tests, the number of true null hypotheses (denoted n_2) out of all N tests can be estimated by a number of procedures (Nettleton et al., 2006). For the present study, the histogram-based procedure first described by Mosig et al. (2001) and simplified by Nettleton et al. (2006) was used with the following algorithm:

Count the P -values in each of the H histogram intervals of width $1/H$

Let y_i be the count in the i th interval for $i = 1, \dots, H$,

Let x_j be the mean of the y_i from $i = j$ to H ,

then,

$n_2 = Hx_{j'}$, where j' = the lowest value of j for which y_j is lower than x_j .

In the present study, H was set to 10 so that bin width was 0.10.

The number of false null hypotheses (denoted n_1) is then obtained by subtraction, $n_1 = N - n_2$. Both n_2 and n_1 are useful statistics for a number of purposes, as will be shown in the following sections.

Proportion of False Positives: Setting Significance Levels for Experiment-Wise Linkage Tests. To control for the multitest situation while retaining power, the proportion of false positive (PFP) criterion (Fernando et al., 2004), also termed aFDR (Mosig et al., 2001), was used to set CWER threshold P -values for declaration of significance at the marker-trait (PFP_M) and sire-marker-trait (PFP_S) levels. Following Mosig et al. (2001) and Fernando et al. (2004), PFP_{Mj(k)}

for the j th marker test within the k th trait was calculated as:

$$\text{PFP}_{\text{Mj}(k)} = (P_{\text{Mj}(k)} n_{2\text{Mk}}) / R_{\text{Mj}(k)}$$

where $P_{\text{Mj}(k)}$ = the CWER P -value of the j th marker test within the k th trait, when the marker tests are ranked by their P -values from lowest to highest within a given trait; $R_{\text{Mj}(k)}$ = the rank number of the j th marker test within the k th trait, and $n_{2\text{Mk}}$ = the number of true null hypotheses for marker tests within the k th trait, estimated as shown above.

Values for the i th sire- j th marker combination within the k th trait [$\text{PFP}_{\text{Sij}(k)}$] were obtained in an exactly analogous manner, except that at the sire-marker level, the number of tests for which null hypothesis is true ($n_{2\text{Sk}}$) represents tests for which either the marker is not in linkage to a QTL or the marker is in linkage to a QTL, but the QTL is homozygous in the sire.

Significance Thresholds. Significance thresholds were calculated separately by traits at the marker level and at the sire-marker level. At the marker level, marker-trait tests having CWER P -values corresponding to $\text{PFP}_{\text{M}} \leq 0.10$ were taken as significant. At the sire-marker level, PFP were calculated across all markers, but only sire-marker-trait tests having CWER P -values corresponding to $\text{PFP} \leq 0.20$ within significant markers were taken as significant.

Estimating Power of the Test

Power of the statistical tests used to determine marker-QTL linkage for the k th trait at the given significance threshold for the marker-trait and sire-marker-trait levels (denoted V_{Mk} and V_{Sk} , respectively) were estimated as observed number of significant tests at each level (n_{OMk} and n_{OSk}) after correction for proportion of false positives, divided by the estimated number of false null hypotheses in the DS at that level ($n_{1\text{M}}$, $n_{1\text{S}}$) obtained as described above:

$$V_{\text{Mk}} = n_{\text{OMk}}(1 - \text{PFP}_{\text{Mk}}) / n_{1\text{Mk}}$$

$$V_{\text{Sk}} = n_{\text{OSk}}(1 - \text{PFP}_{\text{Sk}}) / n_{1\text{Sk}}$$

where the factor $(1 - \text{PFP})$ represents the correction for PFP.

Estimating the Proportion of Heterozygosity at the QTL

At the marker-trait level, a false null hypothesis represents a marker in linkage to a QTL. The proportion of false null hypotheses out of all marker-trait tests for

a given trait ($n_{1\text{Mk}}/N_{\text{Mk}}$) thus represents the proportion of markers in linkage to QTL for the k th trait (denoted Q_{Mk}). At the sire-marker-trait level, the proportion of false sire-marker-trait null hypotheses out of all sire-marker-trait tests for a given trait ($n_{1\text{Sk}}/N_{\text{Sk}}$, denoted Q_{Sk}) represents the joint proportion of markers in linkage to QTL (Q_{Mk}) and of QTL that are heterozygous in the sires (denoted H_k). It follows that $Q_{\text{Sk}} = Q_{\text{Mk}}H_k$, and H_k can readily be estimated as $H_k = Q_{\text{Sk}}/Q_{\text{Mk}}$. This expression is algebraically equivalent to the corresponding expression given in Mosig et al. (2001). As pointed out by Mosig et al. (2001), QTL that are not represented in the sample by at least 1 sire heterozygous at both the marker and the QTL will not be included among the significant markers, so that Q_{Mk} is an underestimate. This, in turn, leads to the H_k being overestimates. Mosig et al. (2001) also presented a procedure for correcting for the bias. However, when they applied this procedure to their data, the bias was close to 10%, and should be even lower for the present DS, because it includes a larger number of sires. Given the negligible bias, the correction procedure was not implemented in the present study.

Allele Substitution Effects

Allele substitution effects were calculated as described (Lipkin et al., 1998), based on shadow-corrected allele frequency estimates and the mean EBV of the pools (Table 4). The effects were calculated for all sire-marker-trait combinations defined as significant on the above PFP_{M} and PFP_{S} criteria.

Estimating the Proportion of QTL Affecting More Than One Trait

In an appreciable number of instances (denoted O_{ij}), a given marker was observed to have significant effects on 2 traits, i and j . On the simplest assumption, this can be taken to reflect an underlying pleiotropic effect of the corresponding QTL in linkage to the marker on the pair of traits. It follows that the proportion of markers affecting trait i that also affect trait j is also an estimate of Q_{ij} , the proportion of QTL affecting i that also affect j .

Let O_{Mij} be the observed number of markers that affect both traits. Because the observed marker effects include false positives, the number of observed marker effects on the 2 traits that represent true effects on both traits is equal to $O_{\text{Mij}}(1 - \text{PFP}_{\text{M}})^2$. On the other hand, because of incomplete power of the experiment, the actual number of markers affecting the 2 traits will be $n_{1\text{Mij}} = O_{\text{Mij}}(1 - \text{PFP}_{\text{M}})^2 / V_i V_j$, where V_i and V_j = the power for detecting marker effects on traits i and j ,

Table 4. Mean EBV of high and low pools by sire¹

Sire	PP (%)		PY (kg/yr)		MY (kg/yr)	
	High	Low	High	Low	High	Low
1 ²	0.173	-0.046	32.9	0.3	979	345
2 ³	0.237	0.020	14.6	-7.2	154	-854
3 ²	0.052	-0.132	32.5	4.6	1,401	227
4 ³	0.208	-0.017	32.6	2.2	846	-326
5 ³	0.247	0.049	35.6	8.8	755	-260
6 ³	0.241	0.009	20.3	-8.4	352	-766
7 ³	0.259	0.067	19.6	-5.7	221	-765
8 ²	0.196	0.001	28.1	1.4	637	-407
9 ³	0.187	-0.058	33.4	0.4	987	-323
10 ²	0.123	-0.061	34.6	2.9	1,142	-137
Mean	0.192	-0.017	28.4	-0.1	747	-327

¹PP = protein percentage; PY = protein yield; MY = milk yield; high, low = number of daughters in the means of high and low pools, respectively.

²Data set 2 (Table 3).

³Data set 3 (Table 3).

respectively. The true proportion of markers affecting trait *i* that also affect trait *j* (Q_{Mij}) will then be given by the proportion of n_{1Mij} among the total number of estimated false null hypotheses involving *i*, (n_{1Mi}): $Q_{Mij} = n_{1Mij}/n_{1Mi}$. Similarly, the true proportion of markers affecting *j* that also affect *i* (Q_{Mji}) will be given by $Q_{Mji} = n_{1Mji}/n_{1Mj}$, where n_{1Mji} and n_{1Mj} are obtained as described above. Because the estimated numbers of false null hypotheses n_{1Mi} and n_{1Mj} can be different for different traits, it follows that Q_{Mij} and Q_{Mji} need not be equal. That is, the proportion of QTL affecting trait *i* that also affect trait *j* need not be the same as the proportion of QTL affecting *j* that also affect *i*. For example, it might be that a small fraction of QTL affecting MY also affect PY, but almost all QTL affecting PY also affect MY. On the same argument, the true proportion of markers affecting 3 traits (*i*, *j*, *k*) will be $n_{1Mijk} = O_{Mijk}(1 - PFP_M)^3/V_i V_j V_k$.

As calculated above, n_{1Mi} represents the total number of markers that affect trait *i*, including those that also affect *j* and *k*, and n_{1Mij} represents the total number of markers that affect traits *i* and *j*, including those that affect *i*, *j*, and *k*. From this, it follows that the number of markers εn_{1Mi} , εn_{1Mij} , and εn_{1Mik} that exclusively affect traits *i*, *j*, or *k* will equal

$$\varepsilon n_{1Mi} = n_{1Mi} - \varepsilon n_{1Mij} - \varepsilon n_{1Mik} - n_{1Mijk}$$

$$\varepsilon n_{1Mij} = n_{1Mij} - n_{1Mijk}$$

$$\varepsilon n_{1Mik} = n_{1Mik} - n_{1Mijk}$$

and similarly for the other traits.

The proportion of markers affecting trait *i* that exclusively affect trait *i*, and trait combinations *ij*, *ik*, and

ijk, are obtained by dividing εn_{1Mi} , εn_{1Mij} , εn_{1Mik} , and n_{1Mijk} , respectively, by n_{1Mi} .

In principle, it would have been preferable to perform the mapping for each trait in an independent set of sires or daughters. This was achieved in part by the separation of daughters for the same sires in DS1 and DS2. But the logistical and analytical problems posed by extending such an approach to the entire DS seem insuperable. Had such an experiment been performed, we can expect that the proportion of QTL showing pleiotropic effects would have been somewhat less than found in the present study.

RESULTS

Sampling and Population Phenotypic Means

The mean size of the sire half-sib families was 2,779 (1,827 to 4,769) for PP and 2,893 (2,135 to 4,769) for PY and MY (Table 5). With target number of daughters in the designated tails of 220 (DS1) and 230 (DS2, DS3), the average proportion selected in each tail was about 0.08. For PY and MY, the proportion of targeted daughters actually sampled was greater in the high pools (0.83) than in the low pools (0.78), indicating higher herd retention and hence positive selection for these traits. For PP, the proportion actually sampled was the same for high and low pools (0.79).

The phenotypic means of the 2 tails for each trait are shown in Table 4. Mean values for the high and low pools varied among the different sires, with nonsignificant negative correlations between PP and PY: $r = -0.523$ ($P = 0.121$) and -0.357 ($P = 0.311$) for the high tail and for the low tail, respectively; significant negative correlation between PP and MY: $r = -0.867$ ($P = 0.001$) and -0.728 ($P = 0.017$) for the high and low tails, respec-

Table 5. Total number of milk-recorded daughters and number of daughters sampled to the high and low pools by sire and trait¹

Sire	n (PP)	PP		n ⁴ (PY and MY)	PY		MY	
		High	Low		High	Low	High	Low
1 ²	3,394	161	170	3,121	197	176	197	176
2 ³	2,403	182	183	2,403	187	192	189	183
3 ²	2,678	169	171	2,135	195	177	195	176
4 ³	3,472	194	193	3,472	190	176	193	173
5 ³	2,418	180	170	2,418	180	168	171	171
6 ³	2,731	197	186	2,731	187	178	182	181
7 ³	2,139	202	205	2,139	201	191	205	188
8 ²	1,827	148	159	2,218	177	183	177	183
9 ³	4,769	197	192	4,769	194	181	197	183
10 ²	1,957	177	181	3,522	197	172	193	173
Mean	2,779	181	181	2,893	191	179	190	179

¹PP = protein percentage; PY = protein yield; MY = milk yield; n = total number of milk-recorded daughters of the sire; high, low = number of daughters in the high and low pools, respectively.

²Data set 2 (Table 3).

³Data set 3 (Table 3).

⁴n is the same for PY and MY, because both traits were sampled in 1998.

tively; and significant positive correlation between PY and MY: $r = 0.869$ ($P = 0.001$) and 0.693 ($P = 0.026$) for the high and low tails, respectively. These values were as expected by the known correlations between the traits, which have been previously found in literature (Sölkner, 1989; Chauhan and Hayes, 1991; Welper and Freeman, 1992). The mean difference between high and low tails for PP was 0.209% (calculated as high tail mean, 0.192, minus low tail mean, -0.017 ; range, 0.184 to 0.245%). Corresponding values for PY and MY were 28.5 kg (21.8 to 33.0 kg) and 1,074 kg (634 to 1,310 kg), respectively. With proportion selected of 0.08 to each tail, each difference is the equivalent of 3.72 standard deviation units (Falconer and Mackay, 1996). Thus, the above values for the mean difference between the high and low tails provide estimates of 0.056%, 7.66 kg, and 288.7 kg for the within-sire standard deviation of EBV values of the daughter groups for PP, PY, and MY, respectively. Assuming female EBV reliability = 1.0, the standard deviation of EBV values within sires will represent about 0.87 of the population standard deviation of EBV (Lipkin et al., 1998), these can now be estimated as 0.065%, 8.84 kg, and 333.4 kg, for PP, PY, and MY, respectively.

Empirical Standard Error

For DS4, SE(Dtail) was calculated across 1,375 sire-marker-trait-tail combinations and was found equal to 0.072 so that $SE(D_{null}) = 0.072/\sqrt{2} = 0.051$. For DS5 and DS6, SE(Dtail) was calculated across 1,338 sire-marker-trait-tail combinations and was found equal to 0.073 so that again $SE(D_{null}) = 0.051$. Independent subpools were not prepared for the samples of DS2 and

DS3. Given that the same methodology was used, we took the average $SE(D_{null}) = 0.051$ obtained in DS4, DS5, and DS6 as the SE(D) to analyze the DS2 and DS3 data as well. This value is somewhat less than the value of 0.056 reported by Bagnato et al. (2008).

The Proportion of False Null Hypotheses Among All Null Hypotheses

A total of 2,563 sire-marker-trait tests and 402 marker-trait tests were implemented in the present study. Table 6 shows the frequency distribution in bins of width 0.10 of CWER P -values by traits, for sire marker, and for marker tests. At both levels, there was an excess of low (significant) P -values, compared with the null hypothesis expectation of 10% of total tests in each bin. This indicates a high proportion of false null hypotheses in the DS, which are generating the excess low P -values. The excess of low P -values was about twice as great at the marker level than at the sire-marker level. This is as expected, because even when a sire is tested at a marker in linkage to a QTL, a significant sire-marker effect can be obtained only if the QTL is present in a heterozygous state as well. At both marker and sire-marker levels, the excess of low P -values was similar for PP and MY but considerably less for PY. This is due to the generally smaller D-values found for PY. The mean absolute magnitude of D-values was 0.056, 0.048, and 0.057 for MY, PY, and PP, respectively.

Heterozygosity at the QTL

Based on the data of Table 6, estimates of the proportion of false null hypotheses at the sire-marker-trait

Table 6. The distribution of comparison-wise error rate (CWER) *P*-values and derived statistics for tests by traits¹

<i>P</i> -value bin	Sire-marker-trait tests						Marker-trait tests					
	PP		PY		MY		PP		PY		MY	
	n	%	n	%	n	%	n	%	n	%	n	%
0.0 to 0.1	200	23.3	134	15.7	192	22.5	60	44.8	37	27.6	59	44.0
0.1 to 0.2	100	11.7	102	12.0	106	12.4	23	17.2	13	9.7	19	14.2
0.2 to 0.3	91	10.6	94	11.0	104	12.2	12	9.0	13	9.7	13	9.7
0.3 to 0.4	69	8.1	70	8.2	78	9.1	8	6.0	17	12.7	8	6.0
0.4 to 0.5	89	10.4	79	9.3	73	8.5	5	3.7	10	7.5	15	11.2
0.5 to 0.6	61	7.1	82	9.6	54	6.3	2	1.5	3	2.2	5	3.7
0.6 to 0.7	78	9.1	86	10.1	70	8.2	6	4.5	15	11.2	7	5.2
0.7 to 0.8	60	7.0	55	6.5	64	7.5	8	6.0	12	9.0	5	3.7
0.8 to 0.9	51	6.0	76	8.9	59	6.9	7	5.2	7	5.2	2	1.5
0.9 to 1.0	58	6.8	73	8.6	55	6.4	3	2.2	7	5.2	1	0.7
n	857	100.0	851	100.0	855	100.0	134	100.0	134	100.0	134	100.0
n ₁	241	28.1	114	13.4	251	29.4	82	61.4	46	34.3	94	70.1
n ₂	616	71.9	737	86.6	604	70.6	52	38.6	88	65.7	40	29.9
PFP	0.035		0.003		0.026		0.125		0.023		0.189	
n ₀	101		12		74		68		21		76	
Power	0.339		0.070		0.229		0.746		0.411		0.728	

¹PP = protein percentage; PY = protein yield; MY = milk yield; n = total number of tests; n₁ or n₂ = estimated number and percentage of false or true null hypotheses, respectively; PFP (proportion of false positive) = critical CWER *P*-values for designated significance thresholds (0.10 for marker-trait tests, 0.20 for sire-marker-trait tests within significant markers); n₀ = observed number of significant effects according to PFP criterion; power = estimated statistical power of the tests after correction for false positives.

level (Q_S) were $n_{1S}/N_S = 0.281$, 0.134, and 0.282, for PP, PY, and MY, respectively. These represent the estimated proportion of markers in linkage to a heterozygous QTL at the level of the individual sire-trait combination. At the marker level, the corresponding Q_M estimates were $n_{1M}/N_M = 0.612$, 0.343, and 0.701, for PP, PY, and MY, respectively. This represents the estimated proportion of markers in linkage to QTL at the individual trait level. The estimates of the proportion of heterozygosity (H_k) at the QTL for the various traits (Q_S/Q_M) were therefore equal to 0.46, 0.39 and 0.40, for PP, PY, and MY, respectively. Average estimated heterozygosity at the QTL was 0.42, which is very close to the value of 0.44 obtained in Mosig et al. (2001). As noted in the methods section, this is a slight overestimate and should be reduced by about 10%.

Critical CWER *P*-Values and Power According to PFP

Table 6 also shows critical CWER *P*-values for declaration of linkage, according to PFP criteria for significance set at a level of 0.10 for marker-trait tests and 0.20 for sire-marker-trait tests within significant markers. Because PFP values are in direct proportion to n_2 , PP and MY had similar critical *P*-values, which were distinctly higher than the corresponding values for PY. Critical *P*-values for PP were comparable to the values reported for this trait by Mosig et al. (2001).

The Number of Significant Marker-Trait Tests

A total of 134 markers covering 25 chromosomes were tested against each of the 3 traits. Thus, there were 402 tests in all, of which 165 (41.0%) were significant at $PFP_M \leq 0.1$ (appendix Table I). Of the markers tested, 30 were not significant for any of the traits. Of the 104 significant markers, 20 were exclusively significant for PP, 2 for PY, and 27 for MY; 6 were exclusively significant for PP and PY, 36 for PP and MY, 7 for PY and MY, and 6 markers were exclusively significant for all 3 traits. Considering the traits individually, 68 markers (50.7%) on 23 chromosomes had a significant effect on PP (i.e., this includes markers significant exclusively for PP and those significant for PP and PY, PP and MY, and PP, PY, and MY), 21 markers (15.7%) on 15 chromosomes had a significant effect on PY, and 76 markers (56.7%) on 23 chromosomes had a significant effect on MY.

Allele Substitution Effects

Allele substitution effect of all sires significant at $PFP_S = 0.20$ within markers significant at $PFP_M = 0.10$ are presented in Table 7. The mean allele substitution effects of the 3 traits were very close in standard deviation units, 0.30, 0.33, and 0.32 for PP, PY, and MY, respectively. The relatively high allele substitution effects are probably a result of the low power of the experiment at the sire-marker level. As it has been shown

Table 7. Allele substitution effect (α) of all sires significant at proportion of false positive (PFP_S) ≤ 0.20 within markers significant at PFP_M ≤ 0.10 ¹

α	Trait		
	PP (%)	PY (kg/yr)	MY (kg/yr)
Absolute			
Mean	0.019	2.9	107
Range	0.01 to 0.04	2.5 to 3.4	65 to 107
Standardized			
Mean	0.30	0.33	0.32
Range	0.16 to 0.66	0.28 to 0.39	0.20 to 0.62
n	103	9	72

¹n = number of significant sire-trait combinations; absolute α = absolute value of the allele substitution effect in trait units; standardized α = allele substitution effect in units of the trait phenotypic standard deviation (standard deviations as estimated from data of Table 4: PP, 0.064%; MY, 333 kg/yr; PY, 8.83 kg/yr); PP = protein percentage; PY = protein yield; MY = milk yield.

previously, when power is low, allele substitution effects are biased upward. This would apply particularly to PY, which generally showed smaller effects than PP or MY. Thus, the apparently equivalent trait allele substitution effects may be a result of the lower power of PY relative to PP and MY.

The Estimated Proportion of QTL Affecting More Than One Trait

Table 8 shows the number of significant marker-trait combinations by pairs of traits and the estimated proportion of QTL that affect the various trait pairs. Based on these values and on the procedures described in Materials and Methods, it can be calculated that, of the estimated 82 markers affecting PP, 8.9% exclusively affect PP, 14.8% exclusively affect PP and PY, 52.4% exclusively affect PP and MY, and 23.9% affect all 3 traits. Thus, 76.3% of markers affecting PP also affect

MY. Of the estimated 46 markers affecting PY, none exclusively affect PY, 26.3% exclusively affect PY and PP, 33.9% exclusively affect PY and MY, and 42.6% affect all 3 traits. Of the estimated 94 markers affecting MY, 16.0% exclusively affect MY, 16.6% exclusively affect MY and PY, 45.7% exclusively affect MY and PP, and 20.9% affect all 3 traits. Thus, 66.6% of markers affecting MY also affect PP. Considering all 112.6 markers estimated as affecting at least one trait, 6.5% affected PP exclusively, 13.3% affected MY exclusively, none affected PY exclusively, 10.7% affected PP and PY exclusively, 13.9% affected MY and PY exclusively, 38.2% affected MY and PP, and 17.4% affected all 3 traits. Thus, 55.6% of QTL simultaneously affected MY and PP (with or without effects on PY), 17.2% affected PP without effects on MY, and 27.2% affected MY without effects on PP.

DISCUSSION

Empirical SE

In the course of the European Union sponsored BovMAS project, of which this study was a part, the use of an empirical estimate of the standard error for selective DNA pooling analysis was introduced (Bagnato et al., 2008). This was used in the present study in place of the estimate of the standard error based on a priori considerations used in previous studies (Lipkin et al., 1998; Mosig et al., 2001). The a priori approach involved a complex calculation based on many assumptions and approximations (e.g., covariance among allele frequency determinations based on densitometry of multi-allelic mixes) but which did not take into account sources of technical error that are not captured by differences among duplicated pools (such as errors in determining cell or DNA concentration of the samples making up the pools, which lead to over- or underrepresentation of an individual cow in the DNA mix of the pool). In contrast, the empirical estimate is based on the observed variation among independent subpools in the same tail. As such, it should include all sources of technical error, whether defined or not. Indeed, the empirical estimate was found equal to 0.051, compared with previous a priori estimates of SE(D) for the same DS of 0.039 to 0.043 (depending on the estimated frequency of the sire alleles among the dams of the daughters). Thus, the empirical estimate was of the same order of magnitude as the a priori estimates, indicating the general validity of the approach. It was, however, about 20% greater, supporting the view that the empirical estimate included aspects of the samples and data that were not included in the a priori calculations. Thus, the use of split pools and empirical estimates of the standard error should be considered as the standard

Table 8. Proportion of QTL affecting pairs of traits¹

Trait		O _{Mij}	n _{1Mij}	Q _{Mij}
i	j			
PP	PY	12	31.7	0.39
PY	PP	12	31.7	0.69
PP	MY	42	62.6	0.76
MY	PP	42	62.6	0.67
PY	MY	13	35.2	0.77
MY	PY	13	35.2	0.37

¹O_{Mij} = observed number of markers having effects on both trait i and trait j. Six markers that were significant for all 3 traits are included in each of the O_{Mij}. The estimated number of such markers, calculated as described in the text, was 19.6. n_{1Mij} = estimated true number of markers having joint effects on traits i and j after correction for false positives and incomplete power; Q_{Mij} = proportion of QTL affecting trait i that also affect trait j, as estimated from n_{Mij}/n_{1M}; PP = protein percentage; MY = milk yield; PY = protein yield.

option for statistical analysis of selective DNA pooling. It should be noted, however, that the empirical estimate is the same for all markers and pools and does not take into account frequency of the sire allele in the dam population and the number of duplicates and replicates of the pool that were genotyped, both of which may differ among markers and pools.

The Number of True and False Null Hypotheses Among All Null Hypotheses

The present study made extensive use of estimates of the number of true null hypotheses (n_2) among all null hypotheses. These were obtained by a histogram-based approach first proposed by Mosig et al. (2001) using an iterative procedure and later simplified to a short algorithm by Nettleton et al. (2006). Evaluation of this procedure in simulations involving QTL mapping (Fernando et al., 2004) and DNA expression analysis (Nettleton et al., 2006) showed that the procedure was effective in estimating n_2 and much simpler to implement relative to a variety of alternative procedures (Nettleton et al., 2006). The estimate of n_2 is primarily of interest for application of the PFP criterion for setting statistical significance levels (Mosig et al., 2001; Fernando et al., 2004). However, once an estimate of n_2 is available, the corresponding n_1 , an estimate of the number of false null-hypotheses is obtained by simple subtraction and can be used to estimate power of the experiment with respect to uncovering marker-QTL linkage (Mosig et al., 2001). As shown in Mosig et al. (2001), and somewhat differently in the present study, estimates of statistical power can be used within the framework of a multiple-sire daughter design to estimate proportion of heterozygosity at the QTL. In the present study, power estimates were further utilized to estimate the proportion of QTL affecting 2 or more traits.

QTL Effects on PP, MY, and PY

As far as PP is concerned, the high estimated proportion of false null hypotheses at the marker level (61.4%, Table 6) derives from the fact that the markers tested were for the most part chosen from QTLR previously shown to affect PP. The estimated 38.6% of true null hypotheses can be attributed to false positive results in the previous studies, chance sampling such that the new sires were homozygous at some of the QTLR identified in previous studies, and new markers added to explore the flanking regions of the previously identified QTLR, or QTLR reported from other populations. Given that the scan was concentrated on QTLR affecting PP, and that the calculations in this study indicate that

three-fourths of markers affecting PP also affect MY, the proportion of false null hypotheses for MY would have been expected to be somewhat less than what was found for PP. Nevertheless, MY showed an even higher proportion of false null hypotheses (70.1%) than PP (Table 6). A simple explanation for this observation was not found. The proportion of false null hypotheses for PY (34.5%) was considerably less than for PP or MY. This is as expected if most of the markers affecting PP and MY are more or less balanced in their effects on the 2 traits. In this case, the net effect on PY is reduced by the negative correlated effects of MY and PP so that detectable signal strength of PY extends to fewer markers than the signal for MY or PP. The generally lower D-values observed for PY compared with MY and PP noted in the results section are attributed to this factor.

The estimated numbers of true marker-QTL linkages (n_{1M} , Table 6) were 82, 46, and 94 for PP, PY, and MY, respectively, with corresponding power after correction for 10% of false positives of 0.75, 0.41, and 0.73. The power for PP is somewhat higher than the value 0.71 (uncorrected for false positives) reported by Mosig et al. (2001). The increase in power in the present study may reflect the use of 10 sires, whereas only 7 sires were surveyed in Mosig et al. (2001), and the fact that the present study was focused on QTLR found in that study.

Averaging over PP taken as trait i and MY taken as trait j , over two-thirds (0.71) of QTL affecting PP also affect MY and vice versa. The proportion of markers affecting PP that also affect PY was 0.39; the corresponding value for MY and PY was 0.37. In the reverse direction, the proportion of markers affecting PY that also affect PP was 0.69; the corresponding value for PY and MY was 0.77. Thus, about one-third of markers that affect PP or MY also affect PY, whereas about two-thirds of markers that affect PY also affect PP or MY. This is plausible. Many QTL affecting PP or MY will not affect PY because of the inverse relationship between MY and PP. In the converse direction, however, we would expect a QTL affecting PY to have effects on MY or PP or both. Thus, the results of the present study are broadly consistent with Viitala et al. (2003) but also support the presence of an appreciable body of QTL that affect MY or PP without major effects on the alternative trait (see below). These may exert their effects through other mechanisms.

Although most markers, and by implication QTL, were estimated to have effects on both MY and PP, it was also estimated that an appreciable proportion (about one-third) of markers affecting MY did not have major effects on PP and vice versa. Thus, these results imply that effects of individual QTL on MY, PP, and PY can differ. This is supported by studies of the effects

of alleles at individual known genes on the 3 traits, MY, PY, and PP.

CONCLUSIONS

In any experiment involving effects of a QTL on a number of traits, some of the markers will present statistical significance for a single trait only. The analytical problem is to distinguish those QTL that are truly limited in their effect to a single trait from those that affect multiple traits but because of limited experimental power of the study present statistical significance for a single trait only. In the present study, a method was developed to correct for incomplete power. Using this approach, estimates of the proportion of markers affecting PP that also affect MY (and vice versa) averaged 0.71. This agrees well with the Viitala et al. (2003) hypothesis. However, the results of the present study also indicate that there may be a proportion of almost 30% of QTL that affect either PP or MY in a major way, presumably via a mechanism other than liquid volume, with only minor effects on the other trait. This agrees very closely with the QTL mapping results of Bagnato et al. (2008) for MY and PP in Brown Swiss dairy cattle and their homologues in the Holstein breed as represented by the main QTL databases.

The estimate of 30% of QTL that affect either MY or PP without effects on the alternative trait is close to the estimate of about one-third (0.32) of QTL affecting PP or MY that also affect PY. Thus, those QTL that affect primarily PP or MY with only minor effect on the other main trait may be the QTL that have significant effects on PY. As expected from the arithmetical relationships among PP, MY, and PY, QTL affecting PY alone were absent from Viitala et al. (2003) and were estimated as absent in the corrected analysis of the present study. The high proportion of QTL estimated as affecting MY and PP with neutral effects on PY should enable PP to be maintained even when selection for PY results in an increase in MY. Assuming a diallelic QTL, average QTL heterozygosity equal to 0.40 as found in the present study implies allele frequencies in the range 0.7 or more for the more frequent QTL allele. This would be consistent with the longstanding selection for MY that has been underway in the Holstein breed for many generations and suggests that, for the most part, alleles with positive effects on MY (and associated negative effects on PP) may already be at moderately high frequency in the breed.

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Appendix 1

Table I. Marker *P*-values, according to chromosome (*Bos taurus* autosome, BTA) and location (cM)¹

BTA	cM	Marker	PP	PY	MY
1	15.428	BMS574	4.5E-01	9.1E-02	4.6E-01
	46.806	BMS2725	4.3E-02*	6.7E-01	9.1E-01
	73.069	BMS4001	2.0E-03*	3.7E-01	5.1E-01
	83.834	BM8246	7.7E-01	9.6E-01	1.8E-01*
	122.094	CSSM19	7.8E-01	6.4E-01	5.4E-02*
	142.244	BMS4044	1.9E-01	9.2E-04*	4.3E-01
	148.212	BMS922	1.1E-01*	2.6E-02	1.2E-01*
2	11.912	TGLA431	6.3E-03*	4.7E-01	3.0E-02*
	38.901	BM3010	7.6E-01	3.6E-01	3.4E-02*
	50.591	BMS1300	8.7E-01	4.7E-02	3.0E-01
	61.725	BMS1126	1.8E-01	4.7E-01	2.7E-02*
	74.547	TEXAN1	8.4E-02*	1.4E-02*	3.2E-01
	96.134	TEXAN4	1.4E-01	7.6E-01	8.3E-01
	115.437	BM2113	1.8E-01	2.8E-01	6.5E-01
3	17.088	INRA006	9.9E-01	7.5E-01	7.0E-01
	32.883	BMS963	4.2E-01	9.1E-01	4.1E-01
	46.041	BM723	1.7E-01	5.9E-01	2.3E-01
	59.360	INRA003	3.8E-01	4.1E-01	1.0E-01*
	67.980	HUJ246	2.9E-02*	6.4E-02	1.1E-01*
	87.331	HUJII77	2.7E-01	1.7E-02*	1.9E-05*
	116.538	BMS896	9.3E-01	1.1E-03*	1.1E-03*
4	28.447	RM188	1.4E-02*	2.3E-01	2.0E-01
	56.596	BM6437	1.2E-01*	1.1E-01	2.0E-01
5	26.429	RM103	2.8E-01	6.6E-03*	1.8E-06*
	56.303	RM500	3.3E-01	2.0E-01	3.7E-01
	80.145	BM1819	7.9E-02*	3.5E-01	7.5E-01
	112.431	ETH2	4.2E-04*	2.2E-02*	4.9E-06*
6	125.048	BMS597	2.1E-01	3.5E-03*	2.6E-02*
	8.053	INRA133	8.7E-01	3.9E-02	4.2E-02*
	35.398	BM1329	2.6E-04*	3.6E-01	1.2E-02*
	43.936	BMS2508	5.6E-04*	9.5E-02	7.3E-02*
	53.724	BM143	9.6E-05*	1.9E-01	5.4E-04*
	57.566	DIK82	2.2E-02*	7.4E-01	4.2E-02*
	63.865	BM4322	2.0E-03*	2.1E-01	7.6E-02*
	67.401	BMS470	5.1E-06*	3.4E-01	9.5E-04*
	72.437	ILSTS097	9.4E-06*	1.6E-01	1.1E-03*
	79.192	RM28	4.9E-06*	2.7E-01	5.6E-03*
	81.961	BM415	5.3E-07*	7.2E-01	4.8E-03*
	91.548	CSN3	8.0E-06*	6.6E-01	4.1E-02*
	97.728	BM4311	5.6E-03*	1.7E-01	9.6E-02*
	101.408	BM8124	8.3E-01	1.1E-01	4.4E-01
	119.048	BMC4203	3.9E-02*	9.4E-02	1.1E-01*
	127.264	BM2320	8.7E-01	2.3E-02*	1.8E-01*
7	0.000	BM7160	6.1E-02*	6.9E-01	8.6E-02*
	16.756	BMS713	1.2E-01*	4.9E-01	3.2E-02*
	25.388	RM6	7.1E-02*	3.1E-01	2.6E-02*
	32.045	IL4	6.6E-03*	6.6E-01	1.5E-01*
	58.552	UWCA20	3.0E-01	8.6E-01	6.7E-01
	72.888	INRA112	9.7E-02*	9.4E-01	5.5E-01
	90.703	BMS1331	3.4E-01	9.3E-01	6.6E-01
	101.117	BM9065	5.3E-02*	5.1E-01	2.0E-01

8	116.629	ILSTS006	1.8E-01	9.6E-01	7.9E-01
	133.809	BMS1247	1.7E-01	5.3E-01	1.9E-01*
	2.684	BMS1864	1.7E-01	2.6E-01	2.8E-01
	21.146	RM372	9.1E-02*	5.6E-03*	2.0E-01
	31.429	BM310	1.2E-01*	7.0E-01	7.2E-01
9	41.603	BMS678	1.0E-01*	2.8E-01	2.9E-01
	63.032	HUJ174	3.9E-03*	3.1E-01	2.9E-02*
	92.729	BM711	7.7E-01	6.7E-01	1.2E-01*
	122.908	BMS836	3.5E-01	6.6E-01	3.7E-02*
	24.139	BM1227	6.6E-01	5.8E-02	8.0E-07*
10	49.996	UWCA9	3.9E-03*	3.2E-01	8.2E-03*
	64.935	BMS1290	1.4E-02*	1.6E-01	1.9E-01*
	90.690	BM4208	2.9E-02*	6.2E-01	1.9E-02*
	24.700	TGLA131	2.1E-01	9.8E-01	1.1E-01*
	35.073	BRN	8.9E-01	4.5E-01	1.5E-01*
11	61.858	TGLA102	6.2E-01	3.2E-01	5.8E-01
	82.223	BMS1318	9.5E-02*	2.6E-01	4.9E-01
	100.013	BMS614	2.3E-01	1.4E-01	1.4E-01*
	19.440	BM716	3.8E-02*	5.3E-02	3.0E-03*
	54.581	BMS1716	1.1E-01*	1.4E-01	2.9E-01
12	81.800	IDVGA3	6.1E-01	6.2E-03*	4.7E-01
	92.179	HUJV174	3.3E-01	6.0E-01	3.6E-01
	112.550	BMS607	3.2E-01	6.9E-01	6.1E-01
	15.119	BM6108	8.4E-02*	8.9E-04*	2.5E-04*
	20.845	BMS2057	3.2E-01	3.3E-02	2.3E-03
13	50.376	BM860	1.8E-02*	6.7E-01	1.1E-02*
	71.289	BM4028	4.5E-01	3.7E-01	6.6E-02*
	5.798	UWCA21	1.4E-01	4.1E-02	6.0E-01
	8.993	TGLA23	1.2E-01*	7.0E-01	4.7E-01
	22.997	BMS1742	3.2E-02*	4.1E-01	2.3E-04*
14	27.604	BMC1222	2.5E-02*	2.2E-01	3.3E-03*
	51.715	HUJ616	2.0E-03*	1.6E-02*	2.1E-02*
	61.841	TGLA381	1.5E-02*	3.9E-01	6.0E-02*
	80.983	BL1071	8.8E-01	3.8E-01	7.4E-01
	0.000	ILSTS039	1.4E-03*	7.6E-01	2.6E-02*
16	17.846	BM1508	6.5E-01	7.7E-01	2.6E-02*
	43.633	RM011	2.7E-01	8.6E-01	4.3E-01
	69.793	BMS947	7.4E-01	2.3E-02*	1.9E-02*
	100.016	BL1036	4.8E-02*	4.7E-01	2.8E-02*
	14.187	HUJ614	1.4E-01	2.4E-01	2.6E-03*
18	30.248	BM4025	5.8E-01	7.3E-02	6.3E-02*
	54.072	CSSM28	1.5E-01	5.2E-02	2.9E-01
	80.005	BM1706	2.8E-01	2.4E-01	4.7E-02*
	88.986	HUJ625	4.9E-01	4.0E-01	3.5E-01
	54.713	ILSTS002	8.4E-01	7.5E-01	8.8E-01
19	16.044	HEL10	1.8E-02*	1.2E-03*	2.2E-01
	43.319	BMS2142	5.1E-04*	8.3E-01	3.5E-01
	70.250	Map2C	2.9E-01	1.7E-01	3.3E-02*
	95.350	RM388	1.9E-01	3.8E-03*	7.3E-02*
	33.377	BMS2461	1.9E-03*	4.0E-02	2.9E-04*
20	56.964	ILSTS72	8.5E-03*	1.6E-01	4.1E-02*
	77.091	UWCA26	7.6E-01	3.6E-01	3.4E-01
	13.520	HEL5	1.6E-03*	2.7E-01	1.2E-01*
	29.870	BM103	2.1E-03*	8.8E-01	4.7E-01
	35.898	BMS2557	2.8E-01	4.4E-01	4.7E-01
21	45.194	ILSTS16	2.1E-01	7.0E-01	4.6E-01
	62.685	TGLA122	2.4E-03*	3.7E-01	3.0E-01
	75.313	BMS743	4.2E-01	3.8E-01	4.2E-01
	2.864	INRA26	3.8E-04*	2.4E-04*	7.7E-03*
	22.305	INRA194	4.4E-02*	1.9E-02*	2.5E-02*
22	48.926	CSSM41	5.4E-02*	4.2E-01	6.9E-01
	79.358	HMH1R	1.2E-01*	8.5E-01	1.0E-02*
	82.930	BM4102	5.9E-03*	8.7E-02	1.7E-01*
	11.825	CSSM5	1.4E-01	1.5E-01	1.5E-01*
	20.661	RM33	4.7E-02*	2.0E-02*	2.6E-01
23	42.949	BMS468	1.4E-01	7.0E-01	3.3E-02*
	52.290	RM185	2.0E-01	7.6E-01	5.1E-01
	56.303	BM7233	9.0E-01	8.0E-01	3.1E-02*
	71.648	BM1905	6.7E-01	2.6E-01	2.6E-01
	14.446	BMS130	2.0E-02*	1.5E-02*	1.5E-04*
25	31.595	BM737	2.0E-04*	3.8E-02	1.6E-02
	46.438	BMS1353	5.4E-02*	4.5E-01	4.5E-01

26	60.476	BM804	2.8E-02*	3.1E-04*	3.0E-01
	74.685	MAF36	4.0E-01	1.4E-01	2.2E-02*
27	0.000	BM3507	7.4E-01	6.1E-01	1.5E-01*
	15.176	RM209	1.4E-04*	1.0E+00	6.2E-01
	45.253	INRA134	2.1E-01	6.8E-01	5.3E-02*
29	55.750	HUJI-13	6.7E-01	3.0E-01	2.8E-01
	0.924	BM4602	6.3E-02*	9.8E-03*	3.2E-01
	21.116	BMC8012	9.0E-02*	3.4E-01	7.3E-01
	24.150	ILSTS89	8.6E-02*	6.8E-01	4.1E-01
	40.163	RM40	7.0E-01	7.6E-01	1.4E-01*
	62.356	BMC1206	6.0E-01	8.8E-01	4.9E-01

¹PP = protein percentage; PY = protein yield; MY = milk yield.

*PFP_M ≤ 0.1.