Combined Use of Quantitative and Molecular Data in Genetic Evaluations for Milk Production of Dual-Purpose Belgian Blue Cows

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Introduction

Origins and evolution of the Ddual-Purpose Belgian Blue Breed (DP-BBB)

During the latter part of the 19th century local Belgian, mostly black and white, dairy cattle were crossed with Shorthorns. The emerging local breed was later called the Belgian Blue Breed because of the blue roan color that is present with all whites and black and whites. These color patterns seems to be inherited from and be a variation in black of the typical Shorthorn colors except that breeders avoided all blacks. In the breed the Shorthorn red and roan still regularly reappear but those animals cannot be registered. At the beginning of the 20th century crossbreeding had stopped and until the 1950s selection was also strongly focused on milk. Milk recording results from this period indicate that these cattle were producing milk at a similar level than did black and whites. The breeding objective was a dualpurpose breed, rectangular, with good format and musculature and good milking yield (4000 kg at 3.5% fat). Between 1950 and 1970 gradually meat production became more important and starting with the males and then the females, preference was given to muscular development. The results of this selection were increased muscle development (shoulder, withers, back, loins, rump) and a tendency to change general morphology, e.g., inclined hindquarters. Two directions of selection appeared. Most breeders pushed for extreme muscling without any consideration for milk production, others kept the initial objective of a dual-purpose animal, still increasing emphasis on meat. In 1974 a decision was made to create two separated lines, Meat Belgian Blue Breed (M-BBB) and Dual-Purpose Belgian Blues (DP-BBB). The later kept a breeding objective for meat, milk and a functional animal, that is easy to manage and adapted to local conditions. DP-BBB animals are today kept in three regions with a majority of animals (around 2000 cows that are registered and under milk recording) in the Walloon Region of Belgium (2/3 of the population), the remaining animals being in the Flemish Region around Brussels and in the extreme north of France where they are called "Bleue du Nord".

Double muscling and the muscular hypertrophy gene mh and its use in DP-BBB

The double muscling condition is known in several breeds. The first description was made in Shorthorns and it seems that BBB have inherited the condition from them. Since 1950 the frequency of double muscling in the BBB breed increased strongly. The double muscling condition has been recognized as being under the control of a major gene but modified by other genes. Charlier et al. [1995] identified the major gene in BBB as being a mutation inactivating myostatin. The M-BBB animal are homozygous for the mutated allele called mh. The situation was recognized as more complicated than expected in DP-BBB. In fact, not all DP-BBB were +/+, some were mh/+ often even mh/mh. After some discussions, whether a DP-BBB animal can carry the mh allele, differences between Walloon, Flemish and French ("Bleue du Nord") breeders appeared, it was decided that all DP-BBB sires have to be genotyped for the allele before they can be registered in order to let people know the status of the sire. Over the years top breeders genotyped their cows too. Rapidly breeders started to use the mh genotype as a tool in selection decision, probably the first use of genotyping molecular data on a large scale in a bovine breed. Presently breeders use mh/mh sires if they want to have more meat, a choice that can be defended, however they also select milk by solely expecting that daughters of +/+ sires produce more milk. This behavior can be resumed as follows:

- conformation is "bad" for milk production;
- presence of mh is "good" for conformation; therefore :
- solely absence of mh is "good" for milk production.

The objective of this study was to show a way to avoid selection bias and to quantify simultaneously effects of the mh allele through a mixed inheritance model that allowed combined use of quantitative and molecular data [e.g., Van Arendonk *et al.*, 1999]. The first results will be shown for milk production but we intend to extend the approach to all economically important traits.

Material and Methods

Quantitative data

The data for the routine genetic evaluation of February 2004 for milk production provided by the Walloon Breeding Association (**AWE**) was used. It consisted in a total of 5,596,037 first, 4,165,776 second and 2,980,205 third lactation test-day records from all dairy and dual-purpose breeds.

Molecular data and estimation of missing numbers of mh allele

In the present status of our research we had only limited genotypings available, 107 sires and 523 cows. Therefore, out of the total of 956,516 animals in the pedigree only 630 animals genotyped. Additional were genotypings are carried out for the moment in the context of a conservation and selection effort in DP-BBB. It is planned that around 2400 additional genotypings from sires, cows, heifers and calves will be available the next 24-month period. However in the context of this study, as the probability that the 636,455 animals (19966 sires and 616,489 cows) from

other breeds carried the mh allele were very low, the mh genotype of these animals was also put to +/+. Therefore a total of 637,085 animals were considered to have a known genotype. In contrast to other studies that were based on estimated genotype probabilities [Israel and Weller, 2002] using e.g. segregation analysis [Elston and Stewart, 1971] we developed an algorithm that estimated missing numbers of mh alleles which is 2 times the probability that an animal carries the mh allele. This value is then a continuous one between 0 and 2, where 0 represents the +/+ and 2 the mh/mh genotype. The main advantages are that you can track alleles much easier than genotypes and if one neglects the parental origin of the allele one can show that the expected number of mh alleles for an non genotyped animal is the average of those of its parents s and d: $E(q|q_s,q_d) = 0.5(q_s + q_d)$. This holds in both directions, therefore the number of mh alleles for a parent p can be estimated from descendants corrected for mates m: $E(q_p|q,q_m) = 2\overline{q} - \overline{q}_m$. Formulas can be rewritten as deviations from an expected average number of alleles for an animal: $E(q|q_s,q_d) = g + 0.5(q_s - g_s) + 0.5(q_d - g_d)$

where g, g_s and g_d are the expected average numbers of mh alleles of animal, sire and dam. These values are the double of the observed mh allele frequency of the corresponding group of animals. Groups were defined by breed composition (BBB vs. others) and birthyears. Estimation can then be done recursively. This gives the following multiple regression

system:
$$\hat{\mathbf{q}}_{\mathbf{X}} = \hat{\mathbf{g}}_{\mathbf{X}} + \mathbf{B} \left(\mathbf{q}_{\mathbf{Y}} - \hat{\mathbf{g}}_{\mathbf{y}} \right)$$
 where

 $\hat{\boldsymbol{q}}_{\boldsymbol{X}}$ denotes the vector of estimated numbers of

mh alleles and $\hat{\mathbf{g}}_x$ the estimated average number of mh alleles for non genotyped animals, \mathbf{q}_y and $\hat{\mathbf{g}}_y$ are the observed numbers

of mh alleles and the estimated expected average numbers of mh alleles for genotyped animals and **B** is the matrix of regression coefficients. Numerical routines were programmed that allowed the joint estimation of $\hat{\mathbf{q}}_{x}$, $\hat{\mathbf{g}}_{x}$ and $\hat{\mathbf{g}}_{y}$. A quadratic function of the number of mh alleles allowed obtaining the probability that the animal is heterozygous for mh from $\hat{\mathbf{h}}_{x} = \hat{\mathbf{q}}_{x}(2 - \hat{\mathbf{q}}_{x})$.

Mixed inheritance model

Based on the official model used in the genetic evaluation in the Walloon region of Belgium [Auvray and Gengler, 2002], a mixed inheritance model [e.g., Van Arendonk *et al.*, 1999] was developed that included two additional regressions. In order to simplify our first computation, we used a single trait and single lactation model in opposition to the official model that is a multitrait-multilactation model. The model used can be written in matrix notation as:

y = Xb + qs + hd + Q(Wt + Za + Zp) + e

where **v** is a vector of milk, fat or protein TD records, **b** is a vector of the usual fixed effects, **q** is a vector of known or estimated numbers of mh alleles, s is the allele substitution effect (replacement of an + allele by an mh allele), **h** is a vector of known or estimated heterozygosity for the mh allele, d is the dominance effect of mh/+, t is a vector of herd*period of calving environmental random regression coefficients, **a** is a vector of additive genetic random regression coefficients, **p** is a vector of permanent environmental random regression coefficient, e is a vector of random residuals, X, W and Z are incidence matrices, **O** is the covariate matrix for the second order Legendre polynomials.

Estimates of allele substitution and dominance effects would have BLUE properties if all genotypes would be know. As a major genotyping effort is underway properties of future estimation will be closer to BLUE.

Sampling errors for regression coefficients were estimated using mixed model conjugate gradient normal equations (Harville,1979). This method allowed to obtain $Var(\hat{s})$ and $Var(\hat{d})$ by solving the appropriate equations. Significance of allele substitution and dominance effects was tested using an approximate t-test with degrees of freedom equal to N – rank(**X**, **q**, **h**) where N is the number of test-day records in a given lactation.

Solving of both systems of equations was done using the PCG algorithm. Once dominance and allele substitution effects were known, average effects of genotypes were computed for the three lactations. Results for fat and protein percentages were obtained using the regular formulas used in breeding value estimation.

Results and Discussion

Current mating strategies in DP-BBB

Table 1 shows the use of genotyped sires on cows having a genotyped grand-sire. The results are expressed relative to the use of a sire on cows having a maternal grand-sire with the same genotype and are corrected for the differences in genotype frequencies in the population. For example, if a +/+ sire is used on a cow having a +/+ grand-sire, he has 0.55 and 0.49 calves from cows with mh/+ or mh/mh. The results in Table 1 seem to indicate that breeders jump from one type to the other with mh/+ sires being the most heavily used on animals of the other types (+/+ and mh/mh maternal grand-sires).

Table 1. Relative use of different genotyped sires

 for recent calves with a known genotyped grand

 sire.

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Sire	М	Maternel grand sire					
	+/+	Mh/+	mh/mh				
+/+	1.00	0.55	0.49				
mh/+	0.72	1.00	0.75				
mh/mh	0.46	0.65	1.00				

Estimation of numbers of mh alleles

In order to show the potential of our method, Figure 1 gives the estimated evolution of the average number of mh alleles that is the double of the allele frequency by birth-year in BBB cattle under milk recording. These estimates correspond to the group effects for BBB over birthyears. The results obtained reflect very well the evolution of the mh allele in this population. Between 1970 and 1990 there is an extremely fast evolution of the allele frequency starting with nearly 0% to reach levels over 90%. After 1980 several pics are visible that can be explained by changes in meat prices and an increased differentiation between DP-BBB under milk recording and M-BBB cattle that were no longer milk recorded. This is especially visible between 1990 and 1995 when a rather strong decline of the mh frequency was observed. This evolution was also expected because of incentive measures registration of DP-BBB increased. In recent years, at least in the Walloon region of Belgium, the frequent use of mh/mh sires created an increase of the frequency of mh.



Figure 1. Evolution of the average number of mh alleles in the BBB population under milk recording.

Effects of the mh allele on milk, fat and protein

Table 2 gives the allele substitution effects for the mh allele. As expected the cows carrying this allele produced less milk, especially in the first lactation. Fat is the milk component that was mostly affected and the only trait that stayed significant over the three lactation at least for the substitution effect. For all traits, the first is more affected than the following lactations. This might be partly an artifact of the single trait model, because mh/mh cows might be selected over time by the elimination of the worst milkers. Additional research using our multitrait multilaction model, that should more resistant to this selection is here required. A certain degree of dominance was estimated. This has the consequence that the mh/+ genotype is closer to the mh/mh than it is to the +/+ (Table 3). This result seems to be in line with observations from the field that consider mh/+ type closer to the mh/mh type for milk production.

Table 2. Allele substitution (s) and dominance effects (d) and sampling error (SE) for milk, fat, protein and the overall Walloon milk index (V \in L = -0.065 x kg milk + 2.37 x kg fat + 5.42 x kg protein).

Trait		Lactation								
		1			2		3			
		Effect	SE	t-value	Effect	SE	t-value	Effect	SE	t-value
Milk	S	-63.2	10.1	6.26 ***	-56.6	13.3	4.26 ***	-24.4	18.0	1.35 NS
	d	-16.8	13.4	1.26 NS	-32.4	17.9	1.81 NS	5.9	24.2	0.24 NS
Fat	S	-3.81	0.42	9.00 ***	-3.67	0.60	6.11 ***	-2.89	0.78	3.69 ***
	d	-1.54	0.56	2.73 **	-1.43	0.80	1.78 NS	-0.51	1.05	0.48 NS
Protein	S	-1.69	0.31	5.51 ***	-0.49	0.53	0.92 NS	-0.38	0.56	0.67 NS
	d	-0.54	0.41	1.31 NS	-0.56	0.72	0.78 NS	-0.15	0.75	0.20 NS
V€L	S	-14.1	2.1	6.87 ***	-7.7	3.3	2.30 *	-7.3	3.7	1.95 NS
	d	-5.5	2.7	2.00 *	-4.3	4.5	0.96 NS	-2.4	5.0	0.48 NS

NS: non significant, *: P > 0.975, **: P > 0.995, ***: P > 0.9995.

Table 3. Estimated differences between the mh/+ and mh/mh genotypes and the +/+ genotype, expressed as average of the first three lactations.

	Milk	Fat%	Fat	Prot%	Protein	V€L
mh/+	-62.5	-0.058	-4.62	0.018	-1.27	-13.8
mh/mh	-96.1	-0.084	-6.91	0.034	-1.70	-19.4

Fat was clearly more affect by the mh allele than was milk or protein yields. As shown in Table 3 the difference between mh/mh and +/+ reached 0.08% percent fat. As milk fat is linked to fat metabolisms and mh/mh animals have less fat deposits the question remains to be answered if lower milk fat is linked to lower bodyfat?

Conclusions and Implications

Current mating strategies in DP-BBB do consider the genotype of the animals. This is a

(still) rare case of direct use of molecular information in the field. This paper proposed a rather straightforward mixed inheritance model to avoid selection bias and to obtain BLUE estimates of allele substitution and dominance effects, together with BLUP estimates for polygenic breeding values. Missing molecular information can be predicted with reasonable accuracy as long as we only try to assess the number of mh alleles. In the near future given the genotyping effort the proportion of missing genotypes for recent cow will go down rapidly. The method presented here has the advantage that it could rapidly be implemented and that it could be the first step towards the regular combined use of quantitative and molecular data in genetic evaluations.

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