Genomic breeding value estimation for novel functional traits in Brown Swiss Cattle

Dissertation
for the Doctoral Degree
at the faculty of Agricultural Science
Georg-August-Universität Göttingen

presented by
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born in Barßel

Göttingen, July 2013

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Date of disputation: 11.07.2013

„So jemand bewirkt, dass dort zwei Halme wachsen,
wo zuvor nur einer gestanden hat,
hat er mehr für sein Vaterland getan,
als ein General, der eine siegreiche Schlacht geschlagen.“

Friedrich der Große (1712 - 1786)

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Genomische Zuchtwertschätzung für neue funktionale Merkmale beim Schweizer Braunvieh

Das erste Ziel dieser Arbeit bestand darin, genetische Parameter und Genauigkeiten von konventionellen Zuchtwerten für eine Auswahl funktionaler Merkmale des Schweizer Braunviehs zu schätzen. Insgesamt wurden 1.799 Kühe auf 40 Schweizer Betrieben phänotypisiert. Die geschätzten Erblichkeiten zeigten gute Übereinstimmungen mit den Ergebnissen anderer Autoren, wobei einige Erblichkeiten am oberen Ende des bekannten Wertebereichs lagen. So etwa der Milchfluss mit $h^2 = 0,42$ oder die Eutertiefe mit $h^2 = 0,42$. Andere Merkmale wie etwa das Melkverhalten mit $h^2 = 0,04$ oder die Tage bis zur ersten Brunst mit $h^2 = 0,02$ zeigten im Vergleich mit der Literatur hingegen eine geringe Erblichkeit mit entsprechend geringer Genauigkeit der Zuchtwerte. Die meisten Verhaltensmerkmale zeigten relativ hohe Erblichkeiten. So etwa das allgemeines Temperament mit $h^2 = 0,38$; die Aggressivität mit $h^2 = 0,12$ oder die Rangordnung innerhalb der Herde mit $h^2 = 0,16$. Das Merkmal Lage der Labien gilt als Indikatormerkmal für Urovagina. Dieses Merkmal wurde hier zum ersten Mal quantitativ genetisch betrachtet und zeigte mit $h^2 = 0,28$ eine moderate Erblichkeit.

Das zweite Ziel dieser Arbeit bestand darin, genetische Parameter und Genauigkeiten konventioneller Zuchtwerte für die Milchinhaltsstoffe auf Ebene der einzelnen Euterviertel zu schätzen. Dazu waren phänotypische Informationen über Fett-, Eiweiß-, Laktose- und Harnstoffgehalt, sowie über den Gehalt an somatischen Zellen (SCS) und über Hyperkeratosen an den Zitzen für jedes Euterviertel der 1.799 Braunviehkühe verfügbar. Im Vergleich mit den vorderen Eutervierteln wies die Milch aus den hinteren Eutervierteln einen signifikant höheren Gehalt an Laktose und einen signifikant geringeren Gehalt an Fett auf. Der Eiweißgehalt der Milch aus den vorderen Eutervierteln war ebenfalls signifikant erhöht gegenüber dem Eiweißgehalt der Milch aus den hinteren Eutervierteln. Beim Harnstoffgehalt, beim SCS und bei den Hyperkeratosen konnten keine signifikanten Unterschiede zwischen den Eutervierteln festgestellt werden. In Bezug auf die genetischen Parameter zeigten die Hyperkeratosen, der Eiweißgehalt und der SCS eine höhere Erblichkeit an den vorderen Eutervierteln. Der Fettgehalt wies hingegen an den hinteren Eutervierteln eine höhere Erblichkeit auf. Die Gehalte von Laktose und Harnstoff zeigten zwischen den Eutervierteln keine systematischen Unterschiede in der Erblichkeit. Die additiv genetischen Korrelationen zwischen allen Eutervierteln lagen für Eiweißgehalt und

Harnstoffgehalt über 0,9. Im Gegensatz dazu waren die additiv genetischen Korrelationen für den SCS zwischen allen Euterviertеln deutlich geringer (< 0,5). Beim Laktosegehalt und beim Fettgehalt waren die additiv genetischen Korrelationen zwischen den beiden vorderen Euterviertеln bzw. zwischen den beiden hintern Euterviertеln deutlich höher, als die additiv genetischen Korrelationen zwischen einem vorderen und einem hinteren Euterviertel. Somit liegt der Schluss nahe, dass es sich bei den vorderen und hinteren Euterviertеln aus genetischer Sicht um unterschiedliche Organe handelt. Der Grund dafür liegt vermutlich in differenzierten Synthesewegen der verschiedenen Milchinhaltsstoffe begründet.

Das dritte Ziel dieser Arbeit war es, genomische Zuchtwerte für die oben beschriebenen funktionalen Merkmale zu schätzen. Dazu standen konventionelle Zuchtwerte und 777k SNP Information von einem Teildatensatz des o.g. Datensatzes aus 1.126 Tieren zur Verfügung. Die Genauigkeit des direkten genomischen Wertes wurde mittels einer fünffachen Kreuzvalidierung mit 10 Wiederholungen abgeschätzt. Die Korrelationen zwischen den de-regressierten Zuchtwerten und dem direkten genomischen Werten lagen bei 0,63 für das allgemeine Temperament, bei 0,73 für das Melkverhalten, bei 0,69 für die Aggressivität, bei 0,65 für die Rangordnung innerhalb der Herde, bei 0,69 für den Milchfluss, bei 0,71 für die Eutertiefe, bei 0,66 für die Lage der Labien und bei 0,74 für die Anzahl von Tagen bis zur ersten Brunst. Wurden nur SNP Marker verwendet, die sich auf dem kleineren 54k Chip befinden, so veränderten sich die Korrelationen zwischen de-regressierten Zuchtwerten und dem direkten genomischen Wert nur minimal. Die Vorhersage der Zuchtwerte für das jüngste Fünftel der Tiere ergab Korrelationen von 0,55; 0,77; 0,73; 0,55; 0,64; 0,59; 0,67 und 0,77 zwischen direktem genomischen Wert und de-regressiertem Zuchtwert für die oben genannten Merkmale. Ferner fand eine neue Methode Anwendung, welche die Genauigkeit des direkten genomischen Wertes unter Berücksichtigung der Korrelation zwischen direktem genomischen Wert und konventionellen Zuchtwert abschätzt. Dabei ergab sich Genauigkeiten von 0,37; 0,20; 0,19; 0,27; 0,48; 0,45; 0,36 und 0,12 für die oben genannten Merkmale. Diese Genauigkeiten sind zwar deutlich kleiner, als die Korrelationen zwischen de-regressiertem Zuchtwert und direktem genomischen Wert, erscheinen unter Berücksichtigung von Erblichkeit und Stichprobenumfang aber realistischer. Bei der Annotation besonders großer SNP Effekte wurden zwei Kandidatengene gefunden, welche möglicherweise einen nennenswerten Einfluss auf die Merkmale allgemeines Temperament und Tage bis zur ersten Brunst besitzen.

Genomic breeding value estimation for novel functional traits in Brown Swiss Cattle

The first aim of this thesis was to estimate genetic parameters and accuracies of breeding values for a set of functional, behavior and conformation traits in Brown Swiss cattle. Data of 1,799 phenotyped Brown Swiss cows from 40 Swiss dairy herds were analyzed taking the full pedigree into account. Data were collected by Swiss partners (FiBL, Frick). Estimated heritabilities were in a similar range as reported in literature, with results at the high end of the reported values for some traits (e.g. milking speed $h^2 = 0.42 \pm 0.06$, udder depth h^2 0.42 ± 0.06) while other traits were of low heritability and heritability estimates are of low accuracy (e.g. milking temperament $h^2 = 0.04 \pm 0.04$, days to first heat $h^2 = 0.02 \pm 0.04$). For most behavior traits we found relatively high heritabilities (general temperament $h^2 = 0.38 \pm 0.07$, aggressiveness $h^2 = 0.12 \pm 0.08$ and rank order in herd $h^2 = 0.16 \pm 0.06$). For position of labia, which is arguably an indicator trait for pathological urovagina and was genetically analysed in this study for the first time, a moderate heritability ($h^2 = 0.28 \pm 0.06$) was estimated.

The second aim of was to estimate genetic parameters and accuracies of breeding values for milk content traits of individual udder quarters in Brown Swiss cattle. Fat, protein, lactose, and urea content, somatic cell score (SCS) and information about hyperkeratosis were available for each udder quarter of the 1,799 cows. The milk of the rear udder quarters was found to have a significantly higher lactose content and a significantly lower fat content than milk of the front udder quarters. The same trend as for fat content was observed for protein content, while no differences between the udder quarters were observed for urea content, SCS and hyperkeratosis. In regard to genetic parameters hyperkeratosis, protein content and SCS showed higher heritabilities in the front udder quarters, fat content had higher heritabilities in the rear udder quarters, and no systematic pattern in heritability was observed for lactose content and urea content. Additive genetic correlations between all udder quarters were above 0.9 for protein and urea content, while they were remarkably low (< 0.5) for SCS. For the traits lactose and fat content the genetic correlations between the two front or the two rear quarters, respectively, were found to be distinctively higher than correlations between one front and one rear quarter, suggesting that the front and the rear udder could be considered as partly genetically different organs. Some of these findings can be explained by differences in the physiological background of the traits.

The third aim of this study was to estimate direct genomic values for the functional traits general temperament, milking temperament, aggressiveness, rank order in herd, milking speed, udder depth, position of labia, and days to first heat in Brown Swiss dairy cattle. Direct genomic values were estimated based on 777k SNP information from 1,126 animals. Accuracy of direct genomic values was assessed by a fivefold cross-validation with 10 replicates. Correlations between de-regressed proofs and direct genomic values were 0.63 for general temperament, 0.73 for milking temperament, 0.69 for aggressiveness, 0.65 for rank order in herd, 0.69 for milking speed, 0.71 for udder depth, 0.66 for position of labia, and 0.74 for days to first heat. Using the information of the 54k SNP only led to marginal deviations in the observed accuracy. Trying to predict the 20% youngest bulls led to correlations of 0.55, 0.77, 0.73, 0.55, 0.64, 0.59, 0.67, and 0.77 for the traits listed above. Using a novel method to estimate the accuracy of a direct genomic value, defined as correlation between direct genomic value and true breeding value, accounting for the correlation between direct genomic values and conventional breeding values revealed accuracies of 0.37, 0.20, 0.19, 0.27, 0.48, 0.45, 0.36, and 0.12 for the traits listed above. These values are much smaller but probably also more realistic given the heritabilities and samples sizes in this study. Annotation of the largest estimated SNP effects revealed two candidate genes affecting the traits general temperament and days to first heat.

1st Chapter

General Introduction

DEVELOPMENT OF DAIRY CATTLE BREEDING PROGRAMS

Since artificial insemination (**AI**) became widely used in practical dairy cattle breeding, all modern dairy cattle breeding programs are based on an AI breeding program as proposed by Skjerevold and Langholz (1964) which is shown in Figure 1. Following Rendel and Robertson (1950) the genetic gain of an AI breeding program can be assessed by the following formula:

$$\Delta G = \frac{\Delta G_{BS} + \Delta G_{CS} + \Delta G_{BD} + \Delta G_{CD}}{L_{BS} + L_{CS} + L_{BD} + L_{CD}}$$

Where ΔG_i is the selection response on the specific pathway bull sire (BS), cow sire (CS), bull dam (BD) and cow dam (CD), and L_i is the corresponding generation interval. The AI breeding program also makes use of BLUP theory (best linear unbiased prediction) as described by Henderson (1964).

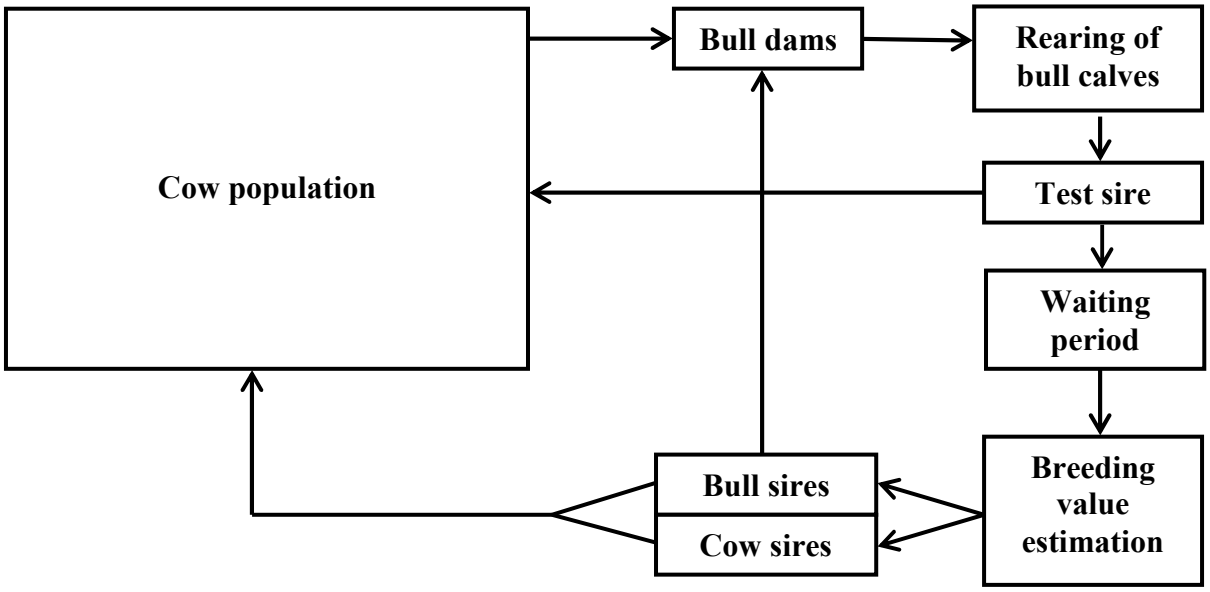

Figure 1: Classical AI dairy cattle breeding program as described by Skjerevold and Langholz (1964). Figure from Swalve and König (2007).

For a typical breeding program the best 2% of the cow population are mated as bull dams to the best 5% of available sires (bull sires). Bull calves out of this mating have to be reared for one year until semen can be collected. A representative sample of the cow population is inseminated with semen of these test sires. Within the waiting period of four

years the daughters of test sires are born (≈ 1 year), are raised (≈ 2 years), and finish their first lactation (≈ 1 year). Based on the first test day records of his daughters a breeding value of the bull can be estimated. Based on these breeding values the decision is made if a sire is culled, or if a sire is widely used in the complete cow population (20% of sires tested will become cow sires; all values from Schaeffer, 2006).

Although the selection intensity on the male path is high and due to a high number of daughters per sire reliabilities are also high, this classical AI breeding scheme has some disadvantages. The first disadvantage is the long generation interval of 5 – 6 years on the bull sire path. The second disadvantage is the possibility of preferential treatment of bull dams by the farmers (Wensch-Dorendorf et al., 2011). A preferential treatment of potential bull dams is justified from an economical point of view for the individual farmer, but will lead to biased breeding values of young sires. One way to prevent these disadvantages is the use of multiple ovulation and embryo transfer in breeding programs (**MOET**) as proposed by Nicholas and Smith (1983). The main characteristic of MOET programs is the use of heifers as bull dams by embryo transfer (**ET**). Thus the generation interval is optimized on all four pathways, but on the other hand, the reliability of breeding values decreases due to the fact that no test day records are available from heifers. This should be prevented by assessing phenotypes in closed nucleus herds where data can be collected with high accuracy (Swalve and König, 2007). In a closed nucleus there is also no risk for preferential treatment. As a result of ET full sisters of bulls are also available and contribute to the breeding value of their brothers. All attempts to implement MOET programs have failed since the method was suggested. One reason is that the intensity of selection within a limited nucleus is always lower than the intensity of selection from the whole population. Furthermore, there is a disease risk within a nucleus which was set up from cows of several herds. Nevertheless some important ideas rose from the discussion of MOET programs for the intensification of classical breeding programs (Swalve and König, 2007). One of these ideas is a system of cooperator herds which will be discussed later.

GENOMIC SELECTION IN DAIRY CATTLE BREEDING

Recently developed techniques make it possible to cover the genome of an animal with up to 700,000 single nucleotide polymorphism (**SNP**) markers. The SNP markers are equally spaced over the genome and in recent years genotyping costs decreased dramatically. In 2006, Schaeffer assumed costs of $500 (≈ 370€) to genotype one animal for 24,072 SNP markers, currently it is possible to genotype an animal for costs of 55€ (Masterrind, 2013) due to decrease in genotyping costs and due to availability of methods to extrapolate SNP information from e.g. 9k information to 54k information (imputation). Meuwissen et al. (2001) first described a method to include genomic information in animal breeding. Schaeffer (2006) made proposals how to implement genomic breeding values (**GEBV**) into dairy cattle breeding (Figure 2).

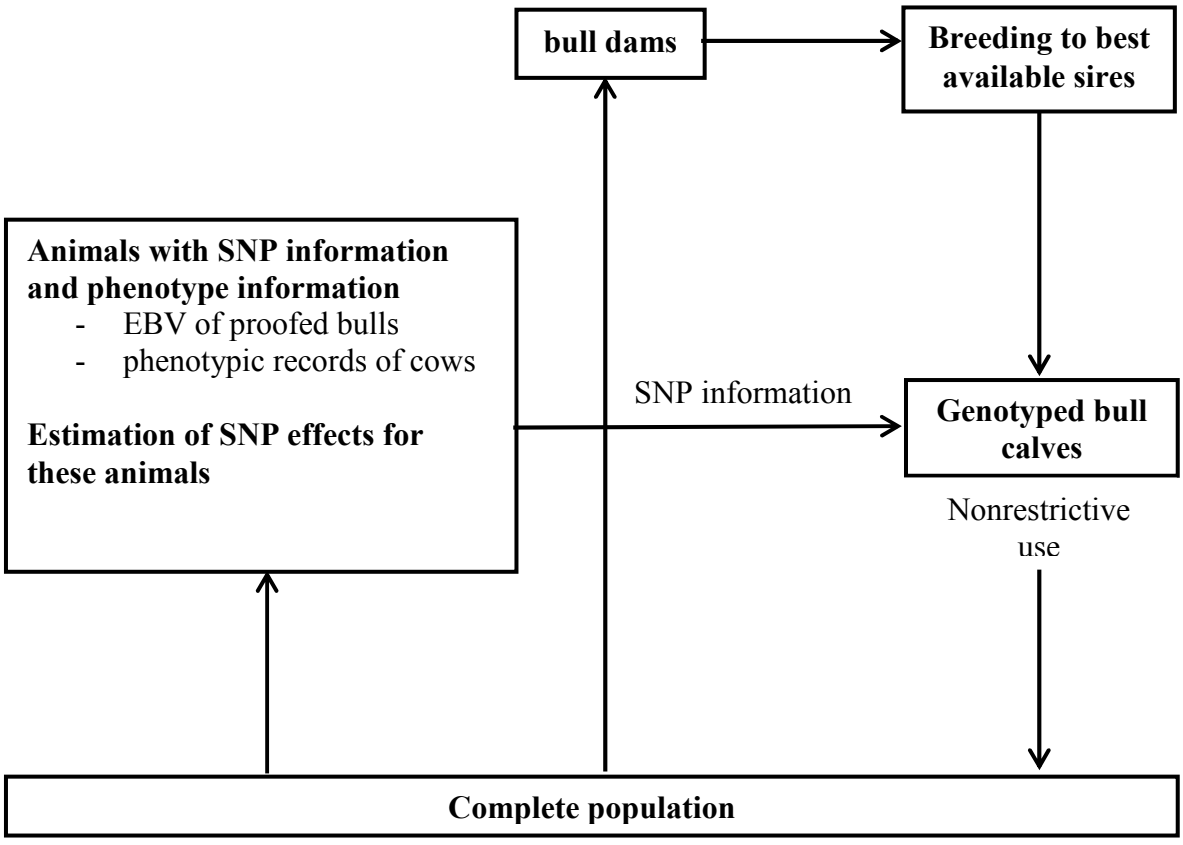

Figure 2: Dairy cattle breeding program with use of SNP information as described by Schaeffer (2006). Figure from Swalve and König (2007).

In a genomic selection scheme SNP effects have to be estimated from a representative sample of animals from the complete population. Schaeffer (2006) claimed to estimate SNP effects on basis of genotyped bulls with accurate EBV based on at least 100

daughters. With decreasing genotyping cost it might also be possible to estimate SNP effects from genotyped cows (possible in cooperator herds) and on basis of their phenotypic records. The SNP information generated from these animals is afterwards used to estimate GEBV for young genotyped bulls. The reliability of GEBV is much higher than the reliability of conventional EBV and so these young bulls can be breed to the complete population without any restrictions as soon as semen can be collected. This reduces generation interval on the bull sire path from 5 - 6 years to just one year. In this scheme, bull dams and bull sires might be preselected on the basis of SNP information, on basis of pedigree, on basis of phenotypic records or on basis of a mixture of these three sources of information.

In recent years, GEBV became well implemented in dairy cattle breeding all over the world and for most economically important breeds. In German Holstein breeding e.g. GEBV became official in August 2010. The last three years showed that GEBV are unbiased (Rensing, 2012). Table 1 gives a comparison between reliabilities (r_{TI}^2) of GEBV and r_{TI}^2 of conventional EBV from pedigree index for the German Holstein population.

Table 1: Reliability (r_{TI}^2) of pedigree index (PI), and genomic breeding value (GEBV) and daughter equivalent for different traits of the German RZG in German Holstein (Rensing 2012).

Breeding value	r_{TI}^2 PI (%)	r_{TI}^2 GEBV (%)	Daughter equivalent
Milk yield (RZM)	31	73	ca. 50, 3 MS[1]
Somatic cells (RZS)	31	76	ca. 85, 3 MS
Conformation (RZE)	28	57	ca. 25
Productive life (RZN)	26	52	ca. 100 1. La[2] + 70 2. La
Daughter fertility (RZR)	25	43	ca. 80, 1. La
Calving ease paternal	33	53	ca. 40 calvings
Calving ease maternal	28	43	ca. 40 calvings
Milking speed (RZD)	24	61	ca. 30
Total merit (RZG)	29	65	

[1] Milk Sample
[2] Lactation

The pedigree index includes all information available from the pedigree for a young bull without daughter records and without SNP information. The GEBV at this stage does not contain information from daughter records either, but it does have pedigree information and additionally SNP information. Table 1 shows that the r_{TI}^2 of pedigree index varies between 25% and 30% for most of the traits. In contrast to this, r_{TI}^2 of GEBV varies on a

higher level between 40 and 76%. This is close to the r_{TI}^2 values which 6 year old bulls with their first daughters in milk achieve, although these young bulls with SNP information are just one year of age (Rensing, 2012). The highest gain in r_{TI}^2 is realized for milk yield and somatic cells (difference of 42% and 45% between r_{TI}^2 of GEBV and EBV). The smallest benefit is realized for daughter fertility and maternal calving eases (difference of 18% and 15% between r_{TI}^2 of GEBV and EBV). The gain of SNP information for breeding value estimation is better described by the number of daughter records which are needed to achieve the same r_{TI}^2 than from genomic information. To get an r_{TI}^2 of 73% (like the GEBV) for milk yield from progeny testing, milk records from three different test days from 50 daughters of a bull are required. This might be possible in short time after the first daughters of a young bull have calved. To get an r_{TI}^2 of 52% for productive life just from daughter records 100 daughters in first lactation and additionally 70 daughters in second lactation are required. This will take a long time and might be actually impossible for some sires. If SNP information is available, an r_{TI}^2 of 52% for productive life is already achieved for a young born bull calf. In this context it has to be mentioned that a GEBV always combines SNP information and several traditional sources of information. For a young sire this is SNP information and information from pedigree, with most weight on SNP information. With an increasing number of daughters in milk, more and more information from daughter records contribute to the GEBV and weight on information from daughter records increases. If just SNP information is considered (Chapter 4) the notation direct genomic value (**DGV**) will be correct.

The possibility to collect data for traits that are difficult to measure with reasonable accuracy within cooperator herds and the potential of genomic selection to estimate breeding values with high r_{TI}^2 for traits with low heritability and for young animals offer the chance to combine these two techniques in a modern breeding program. So in the past several years work was done to assess if there was economic benefit in the use of genomic selection at all, to assess this benefit within cooperator herds, and to develop strategies for finding the best suitable cooperator herds.

COOPERATOR HERDS IN DAIRY CATTLE BREEDING

Functional traits concerning health have become more and more important in dairy cattle breeding in recent years. Data recording on basis of the complete population delivered good results for production traits, but for functional traits an additional assessment of phenotypes is necessary (Bergfeld and Klunker, 2002). This could be done in a system of cooperator herds. Cooperator herds (or test herds) are herds that are contracted to breed cows to young sires and to collect numerous data with high accuracy. The majority of a population in a breeding program with cooperator herds is inseminated by proven bulls. Cooperator herds can thus basically be seen as a pool of nucleus herds. Swalve and König (2007) mention several advantages of cooperator herds. One of these advantages is that the capacity for testing can be ensured even if the proportion of AI decreases. The authors also think that additional phenotypes can be assessed if measurement is very time consuming or cost intensive such as feed intake, energy balance, the level of reproduction hormones or incidences of claw disorders. In a different study, König and Swalve (2006) found that the accuracy of breeding values for the low heritable trait laminitis ($h^2 = 0.14$) could be more than doubled if diagnostics of claw disorders from 50 daughters of a bull were available. This would be much easier in cooperator herds. Another advantage mentioned by Swalve and König (2007) is the fact that less dosses of semen are required to ensure a specific number of daughters with records because herds are contracted to the breeding organization and so farmers are not allowed to sell a cow unless e.g. first lactation is finished. The authors also mention that young sires can be distributed to the herds very efficiently and that there is a better chance to implement methods of molecular biology such as QTL analysis or SNP technique in cooperator herds. There is also a positive marketing aspect if cooperator herds are visited by foreign customers of the breeding organization. Swalve and König (2007) also mention that there might be some concerns, such as a risk of genotype by environment interactions between cooperator herds and the majority of a population and that there is no independent organization for data recording. Nevertheless Swalve and König (2007) state that a system of cooperator herds is essential to use genomic selection efficiently.

As stated by König et al. (2008), herds where additive genetic variance and heritability of the traits under selection are highest should be chosen as cooperator herds. In these herds genetic differences between sires are most obvious. The authors found that heritability and additive genetic variance are significantly positively correlated to herd size and average milk yield of the herd. Heritability and additive genetic variance are further significantly

negatively correlated to average age at first calving and amount of unknown sires. Thus the authors conclude that large farms with high average milk yield and good management (low age at first calving and low amount of unknown sires) should be chosen as cooperator herds. In these herds, the best environments predominate and all cows have the chance to show their full genetic potential.

Estimation of variance components is a very time consuming method and genetic parameters on herd level have to be calculated separately. So Schierenbeck et al. (2011) propose a method to identify cooperator herds by clustering of daughter yield deviations (**DYD**). DYDs are defined as the average phenotypic yield of the daughters of a bull corrected for all fixed environmental effects and for all random genetic effects. They accrue as a co-product in routine breeding value estimation (VanRaden and Wiggans, 1991) and so no extra effort is need for calculation. Schierenbeck et al. (2011) found that daughters with extreme contribute to their sires DYD (either a very high DYD or a very low DYD) belong to herds with high average protein yield, low age at first calving, and low average SCS. Daughters with extreme DYD contribute are also often found in large herds, whereas the number of cows in the herd is not the only criterion if a herd should become cooperator herd. Schierenbeck et al (2011) also state that the low age at first calving reduced generation interval of the breeding program and that large cooperator herds have logistical advantages e.g. in terms of conformation classification or for DNA sampling.

As mentioned above, genotype by environment interactions might be a problem in a system of cooperator herds. König et al. (2005) found drastic differences between residual and permanent environment variance of milk production traits, but also high additive genetic correlations between 0.90 and 0.95 between herds in western and eastern Germany. This is a sign that genotype by environment interactions are small and thus could be neglected. Gernand et al. (2007) analyzed variance components for milk production traits in large and small herds with a focus on cooperator herds and also found no signs for genotype by environment interaction between large and small herds.

IMPLEMENTATION OF GENOMIC PREDICTION IN DAIRY CATTLE BREEDING PROGRAMS

Shortly after the theoretical basis for genomic selection was established by Meuwissen et al. (2001) and after the first SNP chips became available, Schaeffer (2006) examined from a theoretical point of view how genomic selection might influence dairy cattle breeding programs in regard to changes in genetic gain, generation interval, and economical aspects. For the Canadian Holstein industry, Schaeffer (2006) assumed costs of $25 million per year for progeny testing of 500 young Holstein bulls in a conventional AI breeding program. This includes all costs for housing, feeding, semen production, and data recording. Assumed that 20 of these 500 bulls will return to AI, each of these bulls is burdened with costs of $1.25 million. Selection intensity and generation interval on the four different pathways for the Canadian Holstein industry are shown in table 2.

Table 2: Selection intensity (i), accuracy (r_{TI}), and generation interval (L), as well as genetic gain in standard deviations (SD) on the four different pathways for conventional (conv) and genomic (genom) Holstein breeding programs in Canada (Schaeffer, 2006).

Pathway	Selection	i	r_{TI}		L		SD ($i*r_{TI}$)	
			conv	genom	conv	genom	conv	genom
Sire of bull	5%	2.06	0.99	0.75	6.5	1.75	2.04	1.54
Sire of cow	20%	1.04	0.75	0.75	6	1.75	1.05	1.05
Dam of bull	2%	2.42	0.60	0.75	5	2	1.45	1.82
Dam of cow	85%	0.27	0.50	0.50	4.25	4.25	0.14	0.14
Total					21.75	9.75	4.68	4.55

In total, a genetic gain of 4.68 genetic standard deviations (**SD**) is made within one generation, with a sum of generation intervals from all four path ways of 21.75 years in a conventional breeding scheme. So the genetic gain is 0.215 SD per year and costs per SD are $116 million. Due to the high selection intensity and due to the high accuracy of estimates, the sire of bull path has the highest contribution to the genetic gain, but also the longest generation interval. The contribution of dams of cows is the smallest due to low selection intensity and due to low accuracy of EBV.

With the use of genomic selection, the values change fundamentally. The sum of generation intervals on the four pathways decreases from 21.75 years to 9.75 years, because GEBV of good accuracy are available at birth and so animals can be mated with onset of sexual maturity. The genetic gain per generation for a genomic breeding program also decreases slightly from 4.68 SD in a conventional breeding program to 4.55 SD, due

to the lower r_{TI} of genomic breeding values of 0.75 in contrast to 0.99 for conventional breeding values in the sire of bull path. On the other hand, the genetic gain on the dam of bull path increases from 1.45 SD to 1.82 SD in a genomic breeding program. Nevertheless a genomic breeding program will end up with a genetic gain of 0.467 SD per year, which is 2.17 times greater than the genetic gain of 0.215 SD from a conventional breeding program. Schaeffer (2006) calculates the annual costs for a genomic breeding program with $1.95 million, which is just 7.8% of the annual costs of a conventional breeding program. This includes the costs for genotyping young bulls (n = 500) and elite dams (n = 2,000), buying the 20 best young bulls ($100,000 each) and housing these bulls. Schaeffer (2006) also calculated costs of $1.25 million for genotyping an initial sample of 2,500 which is essential for estimation of SNP effects. These costs can be depreciated for several years. In addition, a genomic breeding program causes fewer costs than the annual costs of $25 million of a conventional breeding program, even if the initial sample has to be genotyped every year. Further advantages which Schaeffer (2006) mentioned are the higher quality of semen of young bulls which will result in a lower non return rate and less inbreeding because there is more weight on the dam of bull path. The dams of bulls should also be selected from completely genotyped cooperator herds, where additional phenotypes could be measured. This very early work of Schaeffer (2006) showed that genomic selection has a large potential for increasing genetic gain per year and for reducing costs of dairy cattle breeding.

In contrast to Schaeffer (2006), who estimated the benefit of genomic selection based on the formula of Rendel and Robertson (1950), König et al. (2009) evaluated economic gain of genomic breeding programs for different scenarios based on the gene flow method proposed by Hill (1974). König et al. (2009) also use a more sophisticated model which accounts for two traits (production trait and functional trait) with negative correlation and different heritability in the breeding goal. The scenarios represent different proportion of cows being inseminated by young bulls without daughter records, in order to simulate situations in which not all milk producers might be willing to use bulls without daughters in milk. Results of König et al. (2009) are mostly in line with Schaeffer (2006) concerning the selection response. In both studies selection response is almost doubled. But in contrast to Schaeffer (2006), König et al. (2009) did not find a reduction of costs by 92%, but rather by only 22.4% in the most advanced scenario for genomic selection. The authors also state that genomic selection has an economic advantage in contrast to conventional breeding schemes only if at least 20% of the cows are inseminated by young sires without daughter

records and that costs for genotyping have a minor impact on discounted profit per cow. König et al. (2009) state that the most crucial point for implementation of genomic selection will be whether farmers are willing to inseminate cows with semen of young bulls without any daughter records. König and Swalve (2009) found that the selection response can be increased especially for low heritable functional traits, if additional phenotypic records are available from daughters of young sires coming from cooperator herds.

SCOPE OF THIS THESIS

At the present time functional traits are becoming more and more important in dairy cattle breeding. Great hopes are being placed in the recent developments of genomics for finding economically feasible methods to estimate breeding values for these traits with low heritability. For this reason, it was the overall aim of this work to develop a method of genomic breeding value estimation for some novel functional traits in dairy cattle breeding. This was done using the example of the Brown Swiss population of Switzerland.

In chapter 2, genetic parameters, conventional breeding values and accuracies of conventional breeding values for novel traits concerning behavior/workability, conformation and fertility are estimated in order to deliver input data for a genomic breeding value estimation of these traits.

In chapter 3, genetic parameters for milk content traits on the udder quarter level are estimated in order to identify quarters of higher heritability for some traits, which should thus be given more weight in breeding value estimation or for finding an early indicator for mastitis.

In chapter 4, genomic breeding values were estimated on the basis of 777k SNP information for the traits examined in chapter 2. Commonly used methods to assess the accuracy of gEBV are said to lead to an overestimation of accuracy. So an advanced method that is based on the correlation between conventional breeding value on the one side and the correlation between genomic breeding value and conventional breeding value on the other side was implemented for some of the traits.

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2nd Chapter

Estimation of Genetic Parameters for Novel Functional Traits in Brown Swiss Cattle

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Published in:
Journal of Dairy Science 96: 5954 - 5964

Estimation of Genetic Parameters for Novel Functional Traits in Brown Swiss Cattle

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ABSTRACT

The aim of this study was to estimate genetic parameters and accuracies of breeding values for a set of functional, behavior and conformation traits in Brown Swiss cattle. These traits were milking speed, udder depth, position of labia, rank order in herd, general temperament, aggressiveness, milking temperament and days to first heat. Data of 1,799 phenotyped Brown Swiss cows from 40 Swiss dairy herds were analyzed taking the complete pedigree into account. Estimated heritabilities were in a similar range as reported in literature, with results at the high end of the reported values for some traits (e.g. milking speed $h^2 = 0.42 \pm 0.06$, udder depth $h^2 = 0.42 \pm 0.06$) while other traits were of low heritability and heritability estimates were of low accuracy (e.g. milking temperament $h^2 = 0.04 \pm 0.04$, days to first heat $h^2 = 0.02 \pm 0.04$). For most behavior traits we found relatively high heritabilities (general temperament $h^2 = 0.38 \pm 0.07$, aggressiveness $h^2 = 0.12 \pm 0.08$ and rank order in herd $h^2 = 0.16 \pm 0.06$). For position of labia, which is arguably an indicator trait for pathological urovagina and was genetically analysed in this study for the first time, a moderate heritability ($h^2 = 0.28 \pm 0.06$) was estimated.

Key words: *genetic parameter, accuracy of breeding value, behavior, conformation*

INTRODUCTION

While dairy cattle breeding in the past largely focused on production traits, functional aspects like udder health, milking speed or behavioral traits are becoming more and more important, both from an economic and an animal welfare point of view. In the conventional breeding scheme it is often costly and time consuming to collect sufficient numbers of daughter records for a wide range of functional traits to obtain daughter-based sire predictions with sufficient accuracy. Many of the functional traits have a low heritability, are difficult and/or expensive to measure, and some of them are expressed late in life only. Due to the currently high relevance of functional traits it might be appropriate to consider the possibility of integrating new phenotypic traits related to behavior, health, conformation and fertility in modern dairy cattle breeding programs. For implementing these new traits into a routine breeding value estimation it is important to make phenotyping as cost effective as possible and that recording does not disturb the working routine on a dairy farm. So it was important to us to cover a wide range of traits that were described in different ways (scoring by farmers, scoring by experts, exact measurement of conformation) and also to use simple scales that can be applied by the farmer or at most simple, less expensive tools for measurement.

Heritability of behavioral traits

An important functional trait complex are behavior traits. As shown by Sewalem et al. (2010) behavioral traits and docility of a cow significantly influence her productivity and longevity. The authors found that Holstein cows described as very nervous were 18% more likely to be culled than Holstein cows of average temperament, while cows classified as very calm had a 7% lower risk to be culled than the average. Despite this high importance of behavioral traits in dairy cattle most behavior studies in cattle deal with beef cattle (e. g. LeNeindre et al. 1995; Hoppe et al. 2010). Schutz and Pajor (2001) also state that work on temperament and behavior in dairy cattle is limited. In the last decades, different studies estimated heritabilities for traits that describe behavior and temperament traits of dairy cows. Heritability estimates together with the characteristics of the studies are summarized in Table 1. In general there is a wide range of different traits used to describe behavior and docility of cattle. Heritability estimates of these traits were in a wide range (0.07 - 0.53) but were mostly moderate to low.

Table 1: Overview of estimated heritabilities for behavioral traits in dairy and beef cattle from literature.

Author	Trait	Breed	n	Scale	Measurement done by	heritability
	Dairy cattle behavior					
Dickson et al. (1970)	Milking temperament	Holstein	1,017	1 – 4	Farmer	0.53
	Dominance value	Holstein	921	continuous	Independent person	0.07
Sullivan and Burnside (1988)	Milking behavior	Holstein	18,178	1 – 9	Farmer	0.16
	Aggressiveness at feeding	Holstein	18,178	1 – 9	Farmer	0.11
Rensing and Ruten (2005)	Milking temperament	Holstein	382,500	1 – 9	Farmer	0.07
Sewalem et al. (2011)	Milking temperament (univariate)	Holstein	20,000	1 – 5	Independent person	0.13
	Milking temperament (bivariate)	Holstein	20,000	1 – 5	Independent person	0.20
	Beef cattle behavior					
LeNeindre et al. (1995)	Docility	Limousin	904	6.5 – 17	Independent person	0.22
Nkrumah et al. (2007)	Flight speed	Different cross breeds	302	m/s	Independent person	0.49
Hoppe et al. (2010)	Flight speed score	German Angus	706	1 – 4	Independent person	0.20
	Flight speed score	Charolais	556	1 – 4	Independent person	0.25
	Flight speed score	Hereford	697	1 – 4	Independent person	0.36
	Flight speed score	Limousin	424	1 – 4	Independent person	0.11
	Flight speed score	Simmental	667	1 – 4	Independent person	0.28

Heritability of milking speed

Another important functional trait is milking speed. Sewalem et al. (2010) described the importance of milking speed in two different ways. On the one hand, very slow milking cows disturb the flow of cows through the milking parlor and thus increase production costs due to increased labor costs. On the other hand, Rupp and Boichard (1999) found that very fast milking cows tend to have a high somatic cell score (**SCS**) and so have a higher risk of being affected by mastitis or ultimately to be culled. Hence milking speed is considered as a trait with an intermediate optimum in the range of about 2 kg/min (Winter, 2009). In principle, milking speed of a cow can be measured with two different methods. The first method is a subjective scoring, mostly done by farmers on a scale from 1 – 5 or 1 - 9. Subjectively scored milking speed is a trait with low to moderate heritability (e. g. Rupp and Boichard, 1999, Rensing and Ruten, 2005, Table 2). The other method is an objective measurement in kilogram per minute (during the complete milking event or only in the main milking phase). When objectively assessed, milking speed is a trait with moderate to high heritability (e. g. Lassen and Mark, 2008, Table 2).

Heritability of conformation traits

One conformation trait that influences udder health is udder depth. This trait is defined as the distance between base of udder and hock/ground. Seykora and McDaniel (1985) found a significantly ($p < 0.01$) higher somatic cell count (**SCC**) in cows with low udder depth. This effect is stronger the older a cow is. Most studies that estimated genetic parameters for conformation traits used a scale of 1 – 9 to score udder depth and found moderate heritabilities (e. g. Neuenschwander et al., 2005, Table 2). Heritability of udder depth was found to be higher when objective measurements (in centimeter) were used (Seykora and McDaniel, 1985, Table 2).

A novel trait that is linked to fertility is position of labia (Bühler and Maurer, 2004). As explained by F. Schmitz-Hsu (Swissgenetics, Zollikofen, Switzerland; personal communication) it is suggested that position of labia is highly correlated with urovagina, which in turn has a significant influence on fertility. Gautam and Nakao (2009) found 15.4% of cows from seven herds with clinically relevant urovagina. These cows required more inseminations to get pregnant (5 vs. 2; $P < 0.001$), had more days open (370 vs. 136; $P < 0.001$), and were at higher risk to get endometritis (36.4% vs. 9.2%; $P < 0.001$) than cows without urovagina. Bühler and Maurer (2004) pointed out that more dairy cattle than beef cattle cows showed pathologic position of urovagina. As a Swiss veterinarian

(A. Zürcher, TopAZ Embryotransfer, Lütisburg, Switzerland; personal communication) explained to us, there was a high incidence of cows with pathologic position of labia in Switzerland between 1995 and 2010 due to use of bulls with the corresponding characteristics. Unfortunately, urovagina can only be diagnosed by rectal examination by a veterinarian (Bühler and Maurer, 2004). To prevent impaired fertility resulting from urovagina, a conformation trait that can be scored non-invasively by farmers, or at least by experts, with a high correlation to urovagina may be useful, which could be the trait position of labia.

Heritability of fertility traits

Cow fertility is a trait complex which has a major impact on the overall economic viability of dairy production (de Vries et al. 2005). One important component of fertility is the ability of a cow to return to the reproductive cycle after calving. To describe this, most authors used the time period between calving and first insemination (e. g. Berry et al. 2003; König et al., 2008). This time span is easy to calculate from artificial insemination (**AI**) data, but does not precisely describe the cow's ability to turn back into cycle, since farmers usually skip the first heat for AI. A more exact way to assess the onset of oestrus is by activity measurements (Løvendahl and Chagunda, 2009), or by the progesterone concentration in milk (Royal et al., 2002). While heritabilities for the trait time to first insemination in general were low (< 0.10), direct measurements of the time to first cycle provide a slightly higher heritability (0.10 – 0.18, Table 2).

In this study we reported estimates of variance components and genetic parameters (heritability, repeatability) for a set of traits related to behavior, conformation and functional aspects of Brown Swiss dairy cattle. We also calculated accuracies of estimated breeding values for these traits.

Table 2: Overview of estimated heritabilities for milking speed, udder depth, and interval from calving to first insemination traits in dairy cattle from literature.

Author	Trait	Breed	n	Scale	Measurement done by	heritability
	Measurement of Milking speed					
Meyer and Burnside (1987)	Milking speed	Holstein/Ayrshire	550,422	1 – 5	Farmer	0.21
Boettcher et al. (1998)	Milking speed	Holstein	250,000	1 – 5	Farmer	0.15
Rupp and Boichard (1999)	Milking speed	Holstein	29,284	1 – 5	Farmer	0.17
Ilahi and Kadarmideen (2004)	Milking speed	Brown Swiss, Simental, Holstein	900,628	1 – 5	Independent person	0.25
	Milking speed	Brown Swiss	204,397	Kilogram per minute		0.46
	Milking speed	Simmental	655,989	Kilogram per minute		0.48
Rensing and Ruten (2005)	Milking speed	Holstein	382,500	1 – 9	Farmer	0.10
	Milking speed	Holstein	1,608,800	Kilogram per minute		0.28
Lassen and Mark (2008)	Milking speed free stalls	Holstein	19,347	1 – 9	Farmer	0.29
	Milking speed tie stalls	Holstein	10,843	1 – 9	Farmer	0.35
	Milking speed Baden - Würtemberg	Simmental	26,751	Kilogram per minute		0.37
	Milking speed Bavaria	Simmental	35,555	Kilogram per minute		0.28

Table 2 (continued): Overview of estimated heritabilities for milking speed, udder depth, and interval from calving to first insemination traits in dairy cattle from literature.

Author	Trait	Breed	n	Scale	Measurement done by	heritability
	Measurement of udder depth					
Seykora and McDaniel (1985)	Udder height	Holstein	898	cm		0.52
Neuenschwander et al. (2005)	Udder depth	Holstein	42,807	1 – 9		0.29
Lassen and Mark (2008)	Udder depth free stall	Holstein	19,347	1 – 9		0.37
	Udder depth tie stall	Holstein	10,843	1 – 9		0.46
Interbull (2012)	Udder depth	Brown Swiss	All cows with linear description since 1994	1 - 9	Independent person	0.32

Table 2 (continued): Overview of estimated heritabilities for milking speed, udder depth, and interval from calving to first insemination traits in dairy cattle from literature.

Author	Trait	Breed	n	Scale	Measurement done by	heritability
	Measurement of interval from calving to insemination					
Royal et al. (2002)	Interval calving to first insemination	Holstein	1,080	Days		0.11
	Commencement of luteal activity p.p. (P4 Content)	Holstein	1,212	Days		0.16
Berry et al. (2003)	Interval calving to first insemination	Holstein	8,591	Days		0.02
Andersen-Ranberg et al. (2005)	Interval calving to first insemination	Norwegian Read	1,815,581	Days		0.03
König et al. (2008)	Interval calving to first insemination	Holstein	73,344	Days		0.07
Løvendahl and Chagunda (2009)	Days to first detectable estrus (activity)	Holstein, Jersey, Red Dane	515	Days		0.10 – 0.18
Interbull (2012)	Interval from calving to first insemination	Brown Swiss	All AI Data since 1994	Days		0.04

MATERIAL AND METHODS

Data

To estimate genetic parameters of the different traits, a dataset of 1,799 phenotyped cows was available. These cows belonged to 40 Swiss dairy herds with mean (SD) 32 (14) and range 13 – 70 cows per herd, and they were daughters of 469 sires, with mean (SD) 4 (8) and range 1 - 67 daughters per sire. A subset of these cows was also used in Kramer et al. (2013) to estimate genetic parameters for milk content traits. Phenotypes were collected between November 2009 and April 2011. Every farm was visited five times. All animals present on the visiting day were evaluated. So, not all lactations, but the actual lactations during the collection period were incorporated in the present study. Depending on the trait up to 4 measurements for each trait per cow were available from different lactations. The following eight phenotypes were recorded:

- General temperament was scored by farmers with codes between 1 (very nervous) and 5 (very calm) to describe the temperament that was shown by the cow within the herd environment as described by Juga (1996).
- Milking temperament describes the temperament of a cow during milking. It was also scored by farmers with codes between 1 (very nervous) and 4 (very calm) following Dickson et al. (1970).
- Aggressiveness is a binomial trait scored by farmers to describe if a cow behaves aggressive (0) or untroubled (1) towards herd mates.
- Rank order in the herd was scored by farmers with codes 1 for low rank, 2 for medium rank and 3 for high rank.
- Milking speed describes the time that is needed for milking a cow. Farmers gave a subjective score between 1 (very slow milking) and 6 (very fast milking). This definition of milking speed gives a long term impression of the time that is needed for milking a cow and is so less influenced by milk yield of one specific test day.
- Udder depth is an objective measurement of the distance between udder base and hock in centimeters. Negative values describe an udder base below the hock, high values describe a tight and high udder. Measurement of udder depth was done by experienced persons from a research organization with a simple measurement tool that was constructed for this study.

- Position of labia is a new conformation trait suggested by Bühler and Maurer (2004). It was scored by experienced persons from a research organization with codes of 0 for vertical but oblique labia, 1 for vertical labia, 2 for labia < 50% horizontal, 3 > 50% horizontal and 4 for sunken vulva (a small score is better). Bühler and Maurer (2004) found that cows with position of labia coded with 3 have a significantly lower ($p < 0.05$) NRR75 than cows with code 1 (62.5% vs. 64.6%).
- Days to first heat is the number of days between calving and the first heat after calving that was observed by the farmer. Neither technical equipment nor milk sample analyses (progesterone content) were used for detection of first heat. We did not choose the trait days from calving to first insemination what would have been easily available from AI data, because we thought that days from calving to first heat was closer to physiology. The smaller number of records for this parameter is due to difficulties to detect the first heat just by visual observation.

In general it was one aim of our study to evaluate the utility of data that can be collected by farmers during their routine daily work without any technical devices. E. g. we used a simple scoring system for milking speed and not an exact measurement of milking speed in kg/min, udder depth as a conformation trait that was easy to measure by a self-made tool and explicit no technical devices for detection of heat. Most of the farms were free-stall and only 20% tie-stalls. All tie-stall herds had access to pasture in summer and to an outdoor yard in summer and winter, according to Swiss regulations. This guaranteed a regular, in most cases a daily, interaction between animals. Summary statistics of the available cow traits and independent variables are shown in Table 3.

Before estimation of variance components all records with days in milk > 3 SD above mean and Milk yield per lactation < 3 SD below mean were removed from the dataset. Although the minimum of DIM is 1, only traits like general temperament were scored on the first days post partum, which reflect a general assessment of the animal over a longer period. Measurements were only taken at day 20 or later. The 40 herds cover a wide range of extensively and intensively management representative for the Brown Swiss population in Switzerland. So maximum days in milk (after data editing) was 481 in extensively managed herds and maximum lactation milk yield (after data editing) was 12,180 kg in intensively managed herds. Except for milking speed none of the traits is routinely recorded but just collected within the framework of this study. From the complete pedigree 4,208 animals of interest (phenotyped cows and bulls with high impact on the population)

were extracted, for which estimated breeding values (EBVs) were predicted and the respective accuracies of EBVs were calculated.

Table 3: Minimum value (Min), maximum value (Max), mean value (Mean) and standard deviation (SD) of dependent and independent variables (after editing of raw data) used for estimation of variance components.

Trait	n	Scale	Min	Max	Mean	SD
Dependent variables						
General temperament	2312	Score	1	5	3.53	0.99
Milking temperament	2259	Score	1	4	3.22	0.71
Aggressiveness	2309	Binomial	0	1	0.80	0.40
Rank order in herd	2304	Score	1	3	1.97	0.66
Milking speed	4540	Score	1	6	3.67	1.01
Udder depth	2195	Centimeter above hock	-25	20	5.42	6.25
Position of labia	2232	Score	0	4	1.77	1.00
Days to first heat	1678	Day	12	134	46.26	23.41
Independent variables						
HYS of calving	480					
Age at first calving	4	Class	1	4	2.55	1.18
Lactation number	4	Class	1	4	2.70	1.54
Days in milk		Day	1	481	177	112
Milk yield in lactation		kg	2,290	12,180	6,646	1,218

Model

For estimation of genetic parameters of general temperament, milking temperament, rank order in herd, milking speed, udder depth, position of labia and days to first heat the following linear animal model was used:

$$y_{ijklmn} = HYS_{ijk} + AFC_l + Lakt_m + b_1 DIM_{ijklmn} + b_2 DIM^2_{ijklmn} + b_3 MkgLact_{ijklmn} + a_{no} + p_{no} + e_{ijklmno}$$

For the binary distributed trait aggressiveness, a linear logistic model was fitted (for details see Koenig et al., 2005):

$$\log\left[\frac{\pi_{ijklmn}}{1-\pi_{ijklmn}}\right] = HYS_{ijk} + AFC_l + Lakt_m + b_1 DIM_{ijklmn} + b_2 DIM^2_{ijklmn} + b_3 MkgLact_{ijklmn} + a_{no} + p_{no}$$

In these models the following terms were used:

y_{ijklmn}	dependent variable (milking speed, udder depth, position of labia, rank order in the herd, general temperament, milking temperament, days from calving to first heat)
π_{ijklmn}	probability of a cow to be scored as aggressive
HYS_{ijk}	fixed effect of herd i, year of calving j, season of calving k with i = 1 - 40, j = 1 - 3, k = 1 - 4
AFC_l	fixed effect of age at first calving in months (≤ 28, 29 – 30, 31 – 32, ≥ 33)
$Lact_m$	fixed effect of lactation number (1, 2, 3, ≥ 4)
DIM_{ijklmn}	covariate days in milk
$MkgLact_{ijklmn}$	covariate total milk yield per lactation
$b_1 – b_3$	linear regression coefficients for the covariates
a_{no}	random additive genetic effect of animal n and measurement o
p_{no}	permanent environment effect of animal n and measurement o
$e_{ijklmno}$	random residual effect of cow

For random elements the following distributions were assumed:

$$\mathbf{a} \sim N(0, \mathbf{A}\sigma_a^2)$$

$$\mathbf{e} \sim N(0, \mathbf{I}\sigma_e^2)$$

$$\mathbf{p} \sim N(0, \mathbf{I}\sigma_p^2)$$

Where σ_a^2 is the additive genetic variance, σ_e^2 is the residual variance, σ_p^2 is the variance of permanent environment, **I** is an identity matrix and **A** is the additive genetic relationship matrix. Starting from the complete models above, Proc mixed in SAS (SAS Institute, 2008) was used to identify all factors with significant ($p < 0.05$) influence on the observed trait by stepwise analysis. Only significant effects were included in the model used for

estimation of genetic parameters (Table 4) which is a widely used strategy in multiple trait analyses (c.f. Nielsen et al., 2005; König et al., 2006).

Table 4: Effects selected for evaluated traits: herd, year, season of calving (HYS), age at first calving (AFC), days in milk at day of sampling (DIM), lactation milk yield (MkgLact), additive genetic effect (a), permanent environment effect (p) and residual effect (e). X indicates that the effect was significant ($p < 0.05$) and included in the model for a given trait.

	HYS	AFC	Lact	DIM	DIM^2	MkgLact	a	p	e
General temperament	X		X			X	X	X	X
Milking temperament	X		X			X	X	X	X
Aggressiveness	X		X	X			X	X	
Rank order in herd	X	X	X	X	X	X	X	X	X
Milking speed	X	X	X			X	X	X	X
Udder depth	X	X	X	X	X	X	X	X	X
Position of Labia	X		X				X	X	X
Days to first heat	X					X	X	X	X

We opted to test the effect of MkgLact because according to Schutz and Pajor (2001), a farmer might be e. g. more patient with a high yielding cow of bad temperament that with a low yielding cow of bad temperament. This effect is better described by the amount of milk of the complete lactation, than by the amount of milk of one test day. Estimation of genetic parameters and prediction of EBVs were done with ASReml 3.0 (Gilmour et al., 2009). Genetic parameters for all traits were estimated in univariate analyses.

Heritabilities (h^2) and repeatabilities (w^2) for general temperament, milking temperament, rank order in herd, milking speed, position of labia, and days to first heat were derived from the variance components as:

$$h^2 = \frac{\sigma_a^2}{\sigma_a^2 + \sigma_p^2 + \sigma_e^2}$$

$$w^2 = \frac{\sigma_a^2 + \sigma_p^2}{\sigma_a^2 + \sigma_p^2 + \sigma_e^2}$$

where σ_a^2 is the additive genetic variance, σ_p^2 is the variance of permanent environment and σ_e^2 is the residual variance. Heritabilities and repeatabilities on the underlying liability scale for the binary trait aggressiveness were derived as (for details see Koenig et al., 2005):

$$h^2 = \frac{\sigma_a^2}{\sigma_a^2 + \sigma_p^2 + \frac{\pi^2}{3}}$$

$$w^2 = \frac{\sigma_a^2 + \sigma_p^2}{\sigma_a^2 + \sigma_p^2 + \frac{\pi^2}{3}}$$

Accuracy (r_{TI}) of EBVs was calculated as:

$$r_{TI} = \sqrt{1 - \frac{SE^2}{(1+f)\sigma_a^2}}$$

where SE^2 is the squared standard error of the EBV, f represents the inbreeding coefficient (derived from the diagonal element of the additive genetic numerator relationship matrix) and σ_a^2 is the additive genetic variance of the specific trait estimated from the data. The formula above was based on Misztal and Wiggans (1988) and σ_a^2 was multiplied by $(1+f)$ as suggested by Jamrozik et al. (2000) to account for inbreeding. Pearson`s correlations between EBV were calculated by Proc corr in SAS (SAS Institute, 2008) in order to get an idea of the dependency between the different traits.

RESULTS AND DISCUSSION

Estimates of additive genetic variance, permanent environment variance, residual variance, heritability, reliability and accuracy of EBVs of different groups of animals for the functional traits from univariate analyses are listed in Table 5. In general, heritability estimates were in good accordance with heritability estimates from literature for similar traits (Table 1 and Table 2). Correlations between all breeding values are presented in Table 6. Absolute values of breeding value correlations vary in a wide range between 0.00 for the correlation between rank order in herd and general temperament and 0.49 ± 0.01 for the correlation between rank order in herd and aggressiveness.

Genetic parameters of behavioral traits

We estimated low to moderate heritabilities for the behavioral traits. For general temperament we estimated the highest heritability of 0.38 ± 0.07, milking temperament has a heritability of 0.04 ± 0.04, the binary trait aggressiveness has a heritability of 0.12 ± 0.08, and rank order in herd has a heritability of 0.16 ± 0.06. It has to be considered that for the binary trait aggressiveness the heritability on the observed scale is about half the value obtained on the underlying scale (Dempster and Lerner, 1950). We also found moderate to high repeatabilities of the behavior traits of 0.42 ± 0.03, 0.56 ± 0.03, 0.32 ± 0.04 and 0.32 ± 0.02 for rank order in herd, general temperament, milking temperament and aggressiveness. Heritability estimates for behavioral traits in dairy cattle are rare. Behavioral traits are often subjectively scored and are defined in different ways, on different scales and are scored either by the farmer or by an independent person (Table 1). This fact makes it difficult to compare heritabilities of behavioral traits from different studies. In our study all behavioral traits were scored by the farmers, and thus reflect a long term impression of how a cow behaves in the milking parlor or during handling, so that the random error may be reduced. If on the other hand behavior is scored by an independent person in a unique testing situation (e. g. Hoppe et al., 2010, Sewalem et al., 2011), just the one selective event during the time when scoring was done is described. Consequently heritabilities of dairy cattle behavioral traits (Table 1) scored by farmers are on average higher (heritabilities around 0.20) than of the behavioral traits scored by independent persons (heritabilities around 0.12).

Table 5: Additive genetic variance (σ_a^2), variance of permanent environment (σ_{pe}^2) residual variance (σ_e^2), heritability (h^2), repeatability (w^2), with their standard error (SE), mean accuracy of EBVs for all 4,208 animals in the dataset (rTI all), for phenotyped cows (rTI Cows), and for 30 bulls with at least 10 phenotyped daughters in the dataset (rTI Bulls) from univariate.

Trait	$\sigma_a^2 \pm SE$	$\sigma_{pe}^2 \pm SE$	$\sigma_e^2 \pm SE$	$h^2 \pm SE$	$w^2 \pm SE$	r_{TI} all	r_{TI} Cows	r_{TI} Bulls
General temperament	0.36 ± 0.07	0.16 ± 0.06	0.41 ± 0.02	0.38 ± 0.07	0.56 ± 0.03	0.49	0.67	0.83
Milking temperament	0.02 ± 0.02	0.11 ± 0.02	0.26 ± 0.01	0.04 ± 0.04	0.32 ± 0.04	0.24	0.30	0.47
Aggressiveness	0.62 ± 0.46	1.13 ± 0.46		0.12 ± 0.08	0.32 ± 0.02	0.27	0.34	0.52
Rank order in herd	0.06 ± 0.02	0.09 ± 0.02	0.21 ± 0.01	0.16 ± 0.06	0.42 ± 0.03	0.39	0.51	0.70
Milking speed	0.42 ± 0.06	0.22 ± 0.05	0.36 ± 0.01	0.42 ± 0.06	0.64 ± 0.02	0.53	0.73	0.86
Udder depth	6.94 ± 1.21	4.79 ± 0.94	4.77 ± 0.28	0.42 ± 0.06	0.71 ± 0.02	0.49	0.68	0.83
Position of labia	0.26 ± 0.06	0.05 ± 0.05	0.62 ± 0.03	0.28 ± 0.06	0.33 ± 0.04	0.45	0.62	0.79
Days to first heat	8.33 ± 19.02	95.02 ± 29.84	338.05 ± 24.47	0.02 ± 0.04	0.23 ± 0.05	0.15	0.19	0.31

Table 6: Correlations ± SE between breeding values of the different traits.

Trait	General temperament	Milking temperament	Aggressiveness	Rank order in herd	Milking speed	Udder depth	Position of labia
Milking temperament	0.34 ± 0.01						
Aggressiveness	0.29 ± 0.01	0.28 ± 0.01					
Rank order in herd	0.00 ± 0.02	-0.07 ± 0.02	0.49 ± 0.01				
Milking speed	0.18 ± 0.01	-0.04 ± 0.02	0.02 ± 0.02	0.11 ± 0.01			
Udder depth	-0.24 ± 0.02	-0.19 ± 0.02	0.01 ± 0.02	0.17 ± 0.01	0.13 ± 0.01		
Position of labia	-0.19 ± 0.02	-0.18 ± 0.02	0.07 ± 0.01	0.11 ± 0.01	0.08 ± 0.01	0.32 ± 0.01	
Days to first heat	-0.16 ± 0.02	-0.28 ± 0.02	0.05 ± 0.02	0.13 ± 0.01	-0.05 ± 0.02	0.10 ± 0.01	0.05 ± 0.02

Heritability values for the two apparently similar traits general temperament and milking temperament were very different (0.38 ± 0.07 vs. 0.04 ± 0.04). This is in line with literature, where mostly low heritabilities < 0.10 were reported for milking temperament and moderate heritabilities between 0.12 and 0.25 were found for general temperament (Table 1). Our heritability estimate for general temperament was at the upper end while our heritability estimate for milking temperament was at the lower end of values reported in literature. Hoppe et al. (2010) found a wide range of heritability estimates between 0.11 and 0.36 for the temperament trait flight speed score in different beef cattle breeds (Table 1). The divergence of our results obtained for Brown Swiss cattle from the results of other dairy cattle studies mostly done in Holstein may also reflect a breed difference.

Another trait related to behavior or workability is aggressiveness. In our study we estimated a heritability on the underlying liability scale of 0.12 ± 0.08 for aggressiveness observed as a binary trait, which was at the lower limit compared to values reported in literature. In dairy cattle Sullivan and Burnside (1988) found a heritability of 0.11 for aggressiveness during feeding on the scale of 1 - 9. LeNeindre et al. (1995) estimated a heritability of 0.22 for docility in beef cattle (scale between 6.5 and 17), which can be considered as the opposite of aggressiveness and thus should have similar heritability (Table 1). The different scales (observed scale of 1- 9 or 6.5 – 17, underlying liability scale) and different trait definitions (aggressiveness during feeding, docility, aggressiveness in general) make it difficult to compare our work with literature.

The rank order of a cow in the herd is a trait that reflects the way an animal behaves in a social context. Rank order is a very complex trait and strongly influenced by age and weight of the individual, age of the herd mates and interactions between them (Dickson et al., 1970). As described by Beilharz and Zeeb (1982) whether a cow is horned or dehorned also has an influence on rank order. This makes it difficult to estimate valid genetic parameters and might be a reason for the lack of literature on genetic parameters of rank order. Herds in our study were either horned or dehorned whereby most herds were dehorned. There were at most two of the dehorned herds with single horned cows. So we think that the effect of horned or dehorned cows has no impact on our results. With this background, we estimated a relatively high heritability of rank order in herd of 0.16 ± 0.06.

In our study, behavioral traits were scored by farmers in relatively small herds (32 ± 14 cows/herd). This had both advantages and disadvantages for parameter estimation. On the one hand, farmers handle their cows at least twice a day (during milking). Hence, they know each animal very well and should be able to give a realistic

assessment of a cow's behavior. On the other hand, behavior scoring might be biased by a general assessment of a cow's quality and performance. Farmers are also less experienced in scoring cows on a standardized scale than independent experts are, and so might not use the full range of the scale properly. The high heritability estimates with low standard error we found for behavioral traits suggest that the advantages of farmer's scores on small farms outweigh the disadvantages. Our results seem to suggest that farmers are not more lenient with high yielding cows of bad temperament than with low yielding cows of bad temperament. Results from other studies showed that an objective measurement of behavior leads to much higher heritabilities. Nkrumah et al. (2007) estimated a heritability of 0.49 for flight speed with an objective measurement in m/s. This is almost twice the heritability for flight speed estimated by Hoppe et al. (2010) on a subjective scale of 1 – 4. This indicates that more work should be done to develop methods for objective measurement of behavior phenotypes, possibly yielding higher heritabilities and breeding values of higher accuracy.

Correlations of breeding values for the behavioral traits vary between -0.07 ± 0.02 for the correlation between rank order in herd and milking temperament and 0.49 ± 0.01 for the correlation between rank order in herd and aggressiveness (Table 6). Compared to correlations between other studied traits (e.g. milking speed and position of labia or aggressiveness and udder depth) the correlations between the behavioral traits were high. This shows that always the same trait complex (behavior) was described by the breeding values. But the correlations between these traits were still that low that the traits have to be considered as different traits.

Genetic parameters of milking speed

Another farmer-scored trait on a subjective scale of 1 – 6 in our study was milking speed, for which we estimated a heritability of 0.42 ± 0.06 (Table 5). On the one hand, this heritability is at the high end for subjectively scored milking speed. In other studies this trait showed a low to moderate heritability between 0.10 and 0.25 (e. g. Ilahi and Kadarmideen, 2004, Rensing and Ruten, 2005, Table 2). On the other hand, our heritability estimate is close to heritability estimates from objectively measured milking speed (values between 0.28 and 0.48, Table 2). The discrepancies between our findings and results from literature have different reasons. As mentioned by Ilahi and Kadarmideen (2004) the heritability of a trait that was measured on a continuous scale (kilogram per minute for milking speed) is usually higher than the heritability of a trait measured on a discrete scale,

because information is lost when a continuous measurement is transformed into a few discrete classes. This partly explains the different heritabilities between subjectively scored milking speed and objectively measured milking speed in literature. As mentioned in the context of behavioral traits, farmers are less experienced with scoring and so they might not use the full range of the scale. For milking speed this is supported by Ilahi and Kadarmideen (2004), who pointed out that more than 60% of the cows got the intermediate code 3 for milking speed when scoring was done on a subjective scale of 1 – 5. To prevent this instinctive preference of the mean value by the scorers, a scale of 1 – 6 is used in Switzerland. Swiss farmers are also experienced in scoring milking speed of their cows because breeding value estimation for milking speed in Switzerland is based on these data for years. This might be the reason for the distribution of scores close to normal in our study. This idea is supported by the work of Boettcher et al. (1998), who suggested that the scoring system for milking speed was applied more accurately by the farmers when it was newly introduced. This led to a higher heritability for milking speed in the study of Meyer and Burnside (1987) of 0.21, whereas Boettcher et al. (1998) estimated a heritability of just 0.15 from farmer scored milking speed with the same scoring system and for the same population of cows 11 years later.

Lassen and Mark (2008) found heritabilities of milking speed in tie stalls to be higher than heritabilities of milking speed in free stalls. They explained this finding with the fact that farmers know their cows better in tie stalls resulting in a better differentiation between cows and less pedigree errors due to fewer errors in documentation of AI. Pedigree errors lead to smaller additive genetic variance and therefore to smaller heritability estimates. Better knowledge of the individual cows is also true for small herds as in our study and could consequently also be a reason for the high heritability we estimated. Our findings for milking speed suggest that it is also possible to describe a phenotype on a discrete and subjective scale with high accuracy if the scale is used assiduously. Consequently, the heritability of these traits is also high. The assiduous use of the scale by the farmers is also reflected by the relatively high repeatability of milking speed of 0.64 ± 0.02. (Table 5). This indicates that farmers have the same mean and variance in mind when scoring cows and so gave the same code if a cow was scored a second time. Breeding values for milking speed showed low absolute correlations (0.02 ± 0.02 for aggressiveness to 0.18 ± 0.01 for general temperament) to all other traits of our study (Table 6).

Genetic parameters of conformation traits

The objective measurement of udder depth (in centimeters above hock) in our work may also be a reason for the relatively high heritability of 0.42 ± 0.06 we estimated for this trait (Table 5). On a discrete scoring scale udder depth is a trait of moderate heritability around 0.30 (Table 2). The official Swiss breeding value estimation system (Interbull, 2012) uses a heritability of 0.32 for udder depth (Table 2) scored on a scale of 1 – 9. Seykora and McDaniel (1985) estimated a heritability of 0.52 from exact measurement of udder height. Lassen and Mark (2008) estimated heritabilities for different conformation traits. While most traits scored on a scale of 1 – 9 have moderate heritabilities between 0.20 and 0.30, stature was measured in centimeters and had a heritability of 0.56. Our results and these findings of Seykora and McDaniel (1985) as well as Lassen and Mark (2008) support the statement of Ilahia and Kadarmideen (2004) that objective measurements on a continuous scale lead to higher heritabilities. The continuous scale and exact measurement (centimeters above hock) also lead to the highest repeatability in our study for udder depth of 0.71 ± 0.02 (Table 5).

For position of labia as a second conformation trait, we estimated a heritability of 0.28 ± 0.06 (Table 5). To our knowledge, this was the first study in which genetic parameters for this trait were estimated, so there are no estimates in the literature for comparison. The value is in the same range as heritability estimates for other conformation traits related to connective tissue such as udder attachment, rear udder attachment height, rear udder attachment width or median suspensory of 0.23, 0.28, 0.21, 0.23, respectively (Neuenschwander et al., 2005). The low repeatability (0.33 ± 0.04, Table 5) of the trait position of labia could be due to the high variability of this trait depending on stage of estrus cycle (A. Zürcher, TopAZ Embryotransfer, Lütisburg, Switzerland, personal communication). It was surprising to us that position of labia had a relatively strong breeding value correlation to udder depth of 0.32 ± 0.01 (Table 6). On the one hand a high distance between udder and hock represents a tight udder and high quality connective tissue. On the other hand a high score for position of labia represents a weak connective tissue around labia. Nevertheless, position of labia appears to be an interesting new phenotype and more work should be done to investigate the phenotypic and additive genetic correlation between urovagina and position of labia.

Genetic parameters of fertility traits

The trait number of days from calving to first heat, as a trait that reflects the ability of a cow to come back to the reproductive cycle after calving, was practically not heritable ($h^2 = 0.02 \pm 0.04$). Days to first heat also had the lowest repeatability in our work of just 0.23 ± 0.05 (Table 5). Correlations of days to first heat to other traits were mostly weak (Table 6). Other authors usually used the similar trait interval from calving to first insemination (Table 2) and also estimated low heritabilities between 0.02 (Berry et al., 2003) and 0.11 (Royal et al., 2002). The official Swiss breeding value estimation system (Interbull, 2012) uses a heritability of 0.04 for the interval from calving to first insemination (Table 2). In dairy cattle breeding schemes, dates of calving and AI are available with high reliability and for low costs, so days from calving to first insemination is an easy to calculate trait. At the same time, the date of first insemination is strongly influenced by farm management decisions, when e. g. high yielding cows are inseminated later, cows may be inseminated late because of seasonal calving (Andersen-Ranberg et al., 2005) or because a heat is overlooked. For this reason, the interval from calving to first heat – as used in our study – reflects the cow's ability to turn back into cycle after calving much better than the number of days to first insemination does. Heat detection by visual observation only, as done in our study, is labor intensive and particularly heats during the night might not be noticed. Therefore, other traits that are closer to the physiological background like progesterone concentration in milk (Royal et al., 2002) or activity measured by a technical device (Løvendahl and Chagunda, 2009) can be used to detect heat. Progesterone concentration and activity showed heritabilities around 0.20 (Table 2), a value almost twice the heritability for days to first heat or days to first insemination. It has to be considered that progesterone concentration or activity are just auxiliary traits for the trait of interest (days from calving to first heat). The utility of a correlated trait also depends on the additive genetic correlation between the auxiliar trait and the trait of interest.

Accuracies of breeding values

The differences in heritability and number of measurements were also reflected in accuracies of EBVs ($\mathbf{r_{TI}}$) for the different traits (Table 5). Milking speed was a trait with high heritability, for which the largest number of phenotypic measurements were available (Table 3). This led to the highest r_{TI} of 0.53, if all animals were considered. Bulls with at least 10 daughters in the dataset (n = 26 bulls) have an average r_{TI} for milking speed of

0.86 which is close to the r_{TI} that is achieved by proven sires for some traits. The lowest r_{TI} of 0.15, 0.19 and 0.31 as an average of all animals, cows with phenotypes and bulls with at least 10 daughters in the dataset, respectively, was achieved for days to first heat. This is mostly due to a small heritability with high SE of 0.02 ± 0.04 (Table 5).

For most traits the advantage in r_{TI} of bulls with many daughters is small relative to r_{TI} of EBVs that were based on phenotypes assessed from cows (12% – 19%). This finding might be advantageous when in future functional traits will be implemented in genomic breeding programs. The benefit of genomic selection for low heritable functional traits is expected to outperform the benefit for production traits (König and Swalve, 2009; König et al., 2009). Such a genomic selection scheme will not only be based on genomic data of progeny tested bulls, but also on genomic data of phenotyped cows. Especially for newly introduced phenotypic traits, the training set will predominantly be composed of a limited number of phenotyped cows as in our study.

CONCLUSIONS

Our results showed that it is worthwhile to spend more effort in a more differentiated phenotype collection, because higher heritabilities – and in consequence EBVs of higher accuracy – can already be achieved with a limited number of phenotyped cows. This extra effort can either mean a measurement of sophisticated traits of a trait complex such as behavior, an assiduous use of a subjective scoring system (milking speed on a scale of 1 - 6) or objective measurement of a trait (e. g. udder depth in centimeters above hock). It was shown that EBVs for the considered traits for phenotyped cows and for bulls with several phenotyped daughters can be estimated with reasonable accuracy. We believe that using these EBVs (or quantities derived thereof such as deregressed proofs or daughter yield deviations), derived from a limited number of phenotyped cows in a cost efficient way as quasi-phenotypes might help to establish genomic predictions for new functional traits.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge co-funding from the European Commission, under the Seventh Framework Program for Research and Technological Development, for the Collaborative Project LowInputBreeds (Grant agreement No 222623). However, the views expressed by the authors do not necessarily reflect the views of the European Commission, nor do they in any way anticipate the Commission's future policy in this area.

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3rd Chapter

Estimation of Genetic Parameters for Individual Udder Quarter Milk Content Traits in Brown Swiss Cattle

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Published in:
Journal of Dairy Science 96: 5965 - 5976

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ABSTRACT

The aim of this study was to estimate genetic parameters and accuracies of breeding values for milk content traits of individual udder quarters in Brown Swiss cattle. Fat-, protein-, lactose-, and urea content, somatic cell score (SCS) and information about hyperkeratosis were available for each udder quarter. The milk of the rear udder quarters was found to have a significantly higher lactose content and a significantly lower fat content than milk of the front udder quarters. The same trend as for fat content was observed for protein content, while no differences between the udder quarters were observed for urea content, SCS and hyperkeratosis. Heritabilities for each udder quarter were in the following ranges: fat content 0.09 - 0.14, protein content 0.20 – 0.33, lactose content 0.04 – 0.16, urea content 0.13 – 0.22, SCS 0.18 – 0.32 and hyperkeratosis 0.12 – 0.26. Hyperkeratosis, protein content and SCS showed higher heritabilities in the front udder quarters, fat content had higher heritabilities in the rear udder quarters, and no systematic pattern in heritability was observed for lactose content or urea content. Additive genetic correlations between all udder quarters were above 0.90 for protein and urea content, while they were remarkably low (< 0.60) for SCS. For fat content and lactose content the genetic correlations between the two front or the two rear quarters respectively, were found to be distinctively higher than correlations between one front and one rear quarter, suggesting that the front and the rear udder could be considered as partly genetically different organs. The variability within the udder as such was found to be of low heritability (< 0.10) in general.

Key words: *genetic parameter, accuracy of breeding value, individual udder quarter milk content, within udder variability*

INTRODUCTION

Studies that analyze milk composition traits on the udder quarter level are rare, although measurements of the milk content on an udder quarter level are available from automatic milking systems. Forsbäck et al. (2010) stated that there is a need for knowledge about variation in milk contents on the udder quarter level e. g. in order to enhance udder health. If a cow is suffering from mastitis usually only one udder quarter is affected. Lactose content decreases during early mastitis. With proceeding mastitis somatic cell score (**SCS**) increases. This effect might not be detected on a total milk composite level if only one udder quarter is affected due to dilution. While so far little is known about the genetic basis of the diversity of milk composition between udder quarters, this trait complex may provide some insight into the metabolic stability of a cow. Earlier studies (e. g. Berglund et al. 2007) also showed that one individual udder quarter with altered SCS might be masked in the cow's composite milk. Hence individual quarter measurements of milk composition and quality traits could help to detect mastitis earlier.

Another trait that was recorded for each individual udder quarter separately is hyperkeratosis. Neijenhuis et al. (2001) state that hyperkeratosis is strongly influenced genetically, and breeders could use lesion score as an additional indicator trait to improve udder health. Seykora and McDaniel (1985) found that front teats tend to have slightly stronger hyperkeratosis than rear teats.

One aim of our study was to report means and estimates of (co-) variance components and genetic parameters (heritabilities, repeatabilities, genetic correlations) for measurements of fat-, protein-, lactose-, and urea content, SCS and hyperkeratosis of individual udder quarters in Brown Swiss dairy cows. A second aim was to calculated within udder mean and variance of milk contents and hyperkeratosis and to estimate genetic parameters for these new traits. An increase in within udder variance for one milk content trait might be a sign for an infection of one quarter. In cases where novel traits, such as the quarter individual values discussed her, are included in a genomic breeding scheme, training sets will be composed of a mixture of all progeny tested bulls and performance tested cows available for the respective traits, and the accuracy of the conventional breeding values for the novel traits in the different cohorts are relevant. We thus reported accuracies of estimated breeding values for the quarter individual milk contents and for the mean and the variance of the milk contents.

MATERIAL AND METHODS

Data

A dataset of 1,064 cows phenotyped for fat-, protein-, lactose-, and urea content, as well as SCS was available. 1,403 cows were phenotyped for hyperkeratosis. These cows are a subset of the cows used in M. Kramer et al. (unpublished data) and belong to 40 Swiss dairy herds with mean (SD) 32 (14) and range 13 – 70 cows per herd, and they were the daughters of 469 sires, with mean (SD) 4 (8) and range 1 - 67 daughters per sire. Phenotypes were collected between October 2009 and April 2011. Up to 3 measurements for the content traits per cow and from different lactations were available. For hyperkeratosis up to 4 observations were available for each cow.

We aimed to take one milk sample of each udder quarter per cow close to dry-off for our study. It is known that heritability increases to the end of lactation thus milk samples from the end of lactation should be best suitable to reveal systematic differences between udder quarters. From another research project out of the same framework for some cows a second quarter individual milk sample, taken roughly one week earlier, was available. So up to three measurements of milk contents were available per cow from two different lactations within a time period of 1.5 years. Overall there were 315 cows with repeated measurements in the dataset. It is known that heritability reaches its maximum towards the end of lactation and so this sampling date will provide best data base for estimation of genetic parameters. The samples were used to analyze fat content, protein content, lactose content, urea content and SCS. Fat content, protein content and lactose content (values on a percentage scale) were arcsin transformed (Sokal and Rohlf ,1995) to achieve a normal distribution for estimation of variance components and prediction of EBVs, for lactose content all arcsin values < 11.5 (n = 540) were considered as outliers and neglected. Urea content was measured in mg/100ml. SCS is derived from the somatic cell count (**SCC**) as described by Shook (1993) to achieve a normal distribution:

$$SCS = \log 2\left(\frac{SCC}{100{,}000}\right) + 3$$

Hyperkeratosis was subjectively scored by experienced persons from a research organization. In the framework our study was part of each farm was visited five times and all animals present on the visiting day were evaluated for hyperkeratosis and other

phenotypes. So, not all lactations, but the actual lactations during the collection period were incorporated in the scoring for hyperkeratosis of this study. Thickness and roughness of the teat end callosity was taken into account and teat ends were individually scored for each udder quarter with codes of 0 (No ring), 1 (smooth or slightly rough ring), 2 (rough ring), 3 (very rough ring) and 4 (open lesion or scabs) following Mein et al. (2001). Since only 27 udder quarters were scored with code 4 codes 3 and 4 were combined for all analyses. After this editing most teats were in class 3 and an equal number of teats was in class 1 and class 2.

For some novel analyses we also used the mean and the variance of milk content traits and scores for hyperkeratosis of the four udder quarters within one cow as desired traits. Mean and variance of the quarter specific milk content traits/ hyperkeratosis scores were calculated for each cow and recording event. Mean and variance of milk content traits/hyperkeratosis score were treated as ordinary phenotypes with the variance reflecting the variability in the udder and the mean reflecting the specific milk content of the total composite milk. Out of the Swiss BS population 4,208 relevant animals (phenotyped cows and bulls with high impact on the population) were extracted, for which EBVs were predicted and the respective accuracies of EBVs were calculated. Before estimation of variance components all records with days in milk > 3 SD above mean and Milk yield on test day < 3 SD below mean were removed from the dataset. The 40 herds cover a wide range of extensively and intensively management but are representative for the Brown Swiss population in Switzerland. So maximum days in milk (after data editing) was 478 in extensively managed herds and maximum milk yield (after data editing) close to dry-off was 25.8 kg for single cows in intensively managed herds.

Model

Estimation of genetic parameters and prediction of EBVs were done with ASReml 3.0 (Gilmour et al., 2009). Heritabilities, repeatabilities and covariances between the udder quarters for fat content, protein content, lactose content, SCS and for hyperkeratosis were estimated from multivariate analyses, where each quarter was defined as different traits. Because of problems with convergence for the trait urea content, additive genetic variance, variance of permanent environment and residual variance for this trait were estimated by univariate analyses and additive genetic and phenotypic correlation between udder quarters for urea content were obtained from bivariate analyses. Variance components for mean and

variance of milk contents/hyperkeratosis were also estimated from univariate models. For estimation of genetic parameters the following overall linear animal model was used:

$$y_{ijklmnop} = HYS_{ijko} + AFC_{lo} + Lact_{mo} + b_{1o}DIM_{ijklmnop} + b_{2o}DIM^2_{ijklmnop} + b_{3o}MkgTDay_{ijklmnop} + b_{4o}MkgLact_{ijklmnop} + a_{no} + p_{no} + e_{ijklmnop}$$

In this model the following terms were used:

$y_{ijklmnop}$	dependent variable (individual quarter fat-, protein-, urea-, and lactose content, individual quarter SCS and hyperkeratosis) observed for animal n in udder quarter o and repeated observation p
HYS_{ijko}	fixed effect of herd, year of calving, season of calving with i = 1 - 40, j = 1 - 3, k = 1 - 4
AFC_{lo}	fixed effect of age at first calving in month (≤ 28, 29 – 30, 31 – 32, ≥ 33)
$Lact_{mo}$	fixed effect of lactation number (1, 2, 3, ≥ 4)
$DIM_{ijklmnop}$	covariate days in milk
$MkgTDay_{ijklmnop}$	covariate daily milk yield on the test day next to measurement
$MkgLact_{ijklmnop}$	covariate total milk yield per lactation
$b_{1o} – b_{4o}$	linear regression coefficients for the covariates
a_{no}	random additive genetic effect of the o-th udder quarter of the n-th cow
p_n	random permanent environment effect of cow
$e_{ijklmnop}$	random residual effect of observation p

For multivariate analysis of the quarter individual content traits the distribution of random animal effect was defined as follows:

$$\mathbf{a} \sim N(0, \mathbf{A} \otimes \mathbf{G})$$

and

$$\mathbf{G} = \begin{bmatrix} \sigma^2_{a_{FL}} & & & \\ \mathrm{cov}_{a_{FL\,FR}} & \sigma^2_{a_{FR}} & & \\ \mathrm{cov}_{a_{FL\,RL}} & \mathrm{cov}_{a_{FR\,RL}} & \sigma^2_{a_{RL}} & \\ \mathrm{cov}_{a_{FL\,RR}} & \mathrm{cov}_{a_{FR\,RR}} & \mathrm{cov}_{a_{RL\,RR}} & \sigma^2_{a_{RR}} \end{bmatrix}$$

where $\sigma^2_{a_{FL}}$, $\sigma^2_{a_{FR}}$, $\sigma^2_{a_{RL}}$, and $\sigma^2_{a_{RR}}$, are the additive genetic variances of the udder quarters front left (FL), front right (FR), rear left (RL), and rear right (RR), respectively, $\mathrm{cov}_{a_{X\,Y}}$ is the additive genetic covariance between the udder quarters with *X = FL, FR, RL or RR* and *Y = FL, FR, RL or RR* and $X \neq Y$. **A** is the additive genetic relationship matrix. For multivariate analysis of the quarter individual content traits the distribution of residual error was defined as follows:

$$\mathbf{e} \sim N(0, \mathbf{I} \otimes \mathbf{H})$$

and

$$\mathbf{H} = \begin{bmatrix} \sigma^2_{e_{FL}} & & & \\ \mathrm{cov}_{e_{FL\,FR}} & \sigma^2_{e_{FR}} & & \\ \mathrm{cov}_{e_{FL\,RL}} & \mathrm{cov}_{e_{FR\,RL}} & \sigma^2_{e_{RL}} & \\ \mathrm{cov}_{e_{FL\,RR}} & \mathrm{cov}_{e_{FR\,RR}} & \mathrm{cov}_{e_{RL\,RR}} & \sigma^2_{e_{RR}} \end{bmatrix}$$

Where $\sigma^2_{e_{FL}}$, $\sigma^2_{e_{FR}}$, $\sigma^2_{e_{RL}}$, and $\sigma^2_{e_{RR}}$ are the residual variance of the udder quarter FL, FR, RL, and RR, respectively, $\mathrm{cov}_{e_{X\,Y}}$ is the residual covariance between the udder quarters with *X = FL, FR, RL or RR* and *Y = FL, FR, RL or RR* and $X \neq Y$. **I** is an identity matrix. For multivariate analysis of the quarter individual content traits the distribution of permanent environment effect (the same permanent environment for each udder quarter) was defined as follows:

$$\mathbf{p} \sim N(0, \mathbf{I}\sigma^2_p)$$

Where σ^2_p is the variance of permanent environment and **I** is an identity matrix. For bivariate analyses, in order to estimate covariances between the udder quarters for urea content, the multivariate model was used but matrices **G** and **H** were restricted to two respective udder quarters.

For univariate analysis of urea content and of mean and variance of the milk content traits/hyperkeratosis the linear model as described above was used but the additive genetic effect was simplified to an additive genetic effect of animal instead of additive genetic effect of udder quarter and cow. So the distributions of random effects for univariate analyses were simplified to the following:

$$\mathbf{a} \sim N(0, \mathbf{A}\sigma^2_a)$$

$$\mathbf{e} \sim N(0, \mathbf{I}\sigma^2_e)$$

$$\mathbf{p} \sim N(0, \mathbf{I}\sigma^2_p)$$

Where σ^2_a is the additive genetic variance, σ^2_e is the residual variance, σ^2_p is the variance of permanent environment, **A** is the additive genetic relationship matrix and **I** is an identity matrix. Summary statistics of the independent variables (after data editing) is given in Table 1.

Table 1: Minimum value (Min), maximum value (Max), mean value (Mean) and standard deviation (SD) of independent variables used for estimation of variance components.

Trait	n	Scale	Min	Max	Mean	SD
Herd year season of calving	480					
Age at first calving	4	Class	1	4	2.52	1.17
Lactation number	4	Class	1	4	2.65	1.21
Days in milk		Day	205	478	316	47.89
Milk yield on test day		kg	4	25.8	14.56	3.58
Milk yield in lactation		kg	2,290	12,180	6,646	1,218

Starting from the complete models above, Proc mixed in SAS 9.2 (SAS Institute, 2008) was used to identify all fixed factors which significantly ($p < 0.05$) influence the trait studied by stepwise analysis. Only significant effects were included in the model used for estimation of genetic parameters (Table 2), which is a widely used strategy in multiple trait analyses (c.f. Nielsen et al., 2005; König et al., 2006). For hyperkeratosis we used lactation milk yield (MkgLact) instead of MkgTDay, because we assumed that lesion at teat ends are not primarily influenced by the amount of milk from one specific test day, but more by the average performance of the complete lactation.

Table 2: Effects selected for evaluated traits: herd, year, season of calving (HYS), age at first calving (AFC), days in milk at day of sampling (DIM), milk yield on the nearest test day (MkgTDay) and of the respective lactation (MkgLact), additive genetic effect (a), permanent environment effect (p) and residual effect (e). X indicates that the effect was significant ($p < 0.05$) and included in the model for a given trait.

	HYS	AFC	Lact	DIM	DIM^2	Mkg TDay	Mkg Lact	a	p	e
Fat content	X	X	X	X	X			X	X	X
Protein content	X		X	X		X		X	X	X
Lactose content	X	X	X			X		X	X	X
Urea content	X	X	X	X	X			X	X	X
SCS	X		X			X		X	X	X
Hyperkeratosis	X		X	X	X		X	X	X	X

To test for differences in hyperkeratosis, fat content, protein content, lactose content, urea content and SCS between the four udder quarters, a paired t-test with Proc mixed in SAS (SAS Institute, 2008) was used with the models shown in Table 2 for the specific trait. The only modification of the models in Table 2 was that random additive genetic and permanent environment effects were replaced by a fixed effect for udder quarter.

Thus it was possible to detect differences of milk contents and hyperkeratosis between udder quarters. Heritabilities (h^2) and repeatabilities (w^2) were derived from the variance components as:

$$h^2 = \frac{\sigma_a^2}{\sigma_a^2 + \sigma_p^2 + \sigma_e^2}$$

$$w^2 = \frac{\sigma_a^2 + \sigma_p^2}{\sigma_a^2 + \sigma_p^2 + \sigma_e^2}$$

where σ_a^2 is the additive genetic variance, σ_p^2 is the variance of permanent environment and σ_e^2 is the residual variance. Accuracy (r_{TI}) of EBVs was calculated as:

$$r_{TI} = \sqrt{1 - \frac{SE^2}{(1+f)\sigma_a^2}}$$

where SE^2 is the squared standard error of the EBV, f represents the inbreeding coefficient (derived from the diagonal element of the additive genetic relationship matrix) and σ_a^2 is the additive genetic variance of the specific trait. The formula above is based on Misztal and Wiggans (1988) and σ_a^2 is multiplied by $(1+f)$ as described by Jamrozik et al. (2000) to account for inbreeding.

RESULTS AND DISCUSSION

Phenotypic differences between the udder quarters

In Table 3 mean values for individual udder quarter milk content traits are shown. Fat content of the two front quarters was significantly higher than fat content of the two rear quarters ($p < 0.05$). The same tendency was observed for protein content, although the difference between rear left quarter and the two front quarters was not statistically significant. For lactose content this relation was the other way round, lactose content of

rear udder quarters being significantly higher than of front udder quarters ($p < 0.05$). Concentration of urea did not differ between the four udder quarters and for SCS no clear tendency was observed either.

Table 3: Mean values (solutions of a mixed model) for milk composition traits and hyperkeratosis for the udder quarters front left (FL), front right (FR), rear left (RL) and rear right (RR) within each of the 6 traits.

Trait	n	Scale	Mean FL	Mean FR	Mean RL	Mean RR	SD
Fat content	5275	percentage	3.73^{a}	3.73^{a}	3.49^{b}	3.51^{b}	0.04
Protein content	5244	percentage	4.04^{a}	4.05^{a}	$4.01^{a,b}$	3.99^{b}	0.02
Lactose content	5259	percentage	4.56^{a}	4.48^{b}	4.61^{c}	4.60^{c}	0.02
Urea content	5232	mg/100ml	21.94^{a}	21.71^{a}	22.06^{a}	21.88^{a}	0.20
SCS	5338	log SCS	3.14^{a}	3.30^{b}	3.12^{a}	3.09^{a}	0.05
Hyperkeratosis	8645	Code 0 – 3	$0.93^{a,b}$	0.96^{b}	$0.94^{a,b}$	0.91^{a}	0.02

^{a,b,c}: Different letters describe significant differences within one trait between the four udder quarters ($p < 0.05$)

These results are in line with the ones reported by Berglund et al. (2007). These authors also found significantly higher fat content in milk of the front quarters ($p < 0.05$) and significantly higher lactose content in milk of the rear quarters ($p < 0.05$). In contrast to our work, Berglund et al. (2007) did not observe the difference between the front and rear quarters in protein content. The pattern observed for the fat-, protein- and lactose contents presumably is due to the role of lactose in osmotic regulation. As explained by von Engelhard and Breves (2004), lactose is the main osmotic factor of milk. The higher concentration of lactose in the rear quarters causes more water to diffuse into the milk of these quarters. This is supported by Forsbäck et al. (2010), who found that rear quarters produce more milk than front quarters. The increased secretion of water causes an increased dilution of the synthesized and secreted quantities of fat and protein in the rear udder quarters.

For hyperkeratosis we found no systematic differences between front and rear udder quarters. This is in contrast to findings of Seykora and McDaniel (1985), who found that front teats tend to have slightly higher lesion scores than rear teats. These authors also found that cows with high scores for hyperkeratosis have significant higher SCC ($p < 0.05$). Neijenhuis et al. (2001) assumed that the rough surface of teats with high scores for hyperkeratosis provides a good environment for pathogenic bacteria and prevents successful disinfection of teats. High hyperkeratosis scores are a result of thick callosity.

The thick callosity expands the teat canal and so bacteria can get into the mammalian gland more easily. In contrast to Seykora and McDaniel (1985), Chrystal et al. (1999) found no significant influence of hyperkeratosis on SCS. Breen et al. (2009) found cows with mild and moderate hyperkeratosis have lower risk of having a SCC ≥ 200,000 cells/ml than cows with high or low hyperkeratosis. So from literature it cannot be concluded, if a low or a moderate lesion score is positive or if the lesion score has an influence on SCS at all.

Genetic parameters for mean milk contents

Variance components, heritability and repeatability for mean and variance of fat-, protein-, lactose-, and urea content, SCS and hyperkeratosis are presented in Table 4. Compared to literature, our heritability estimates for mean fat content of 0.10 ± 0.06, mean protein content of 0.21 ± 0.10 and mean lactose content of 0.08 ± 0.07 were low. This was also reflected in low r_{TI} values close to 0.30 for the milk content traits. It has to be considered, that our estimates were based on just one or two milk samples that were taken at the end of lactation. In contrast to that other studies (e. g. Yin et al., 2012) or routine breeding value estimations were based on random regression test day models with approximately 10 milk samples from all days in milk (**DIM**) between 5 and 365. The relatively low repeatabilities for mean milk content traits in our study (Table 4) of values around 0.4 also reflect the high gain of information that additional measurements from different DIM would provide. Our heritability for mean urea content of 0.19 ± 0.08 was in line with estimates from Yin et al. (2012), who estimated a heritability of close to 0.13 during the complete lactation and with results from Stamer et al. (2011), who estimated heritabilities between 0.10 and 0.22 for urea content in first and second lactation. As shown by Yin et al. (2012), milk urea content is a trait with a small variation across the lactation compared to the other milk contents. So additional measurements of urea content provide little additional information and one measurement appears sufficient to estimate a realistic heritability for urea content in milk.

Heringstad et al. (2006) estimated a heritability for SCS of 0.08 for one milk sample of first lactating cows, Rupp and Boichard (1999) found a heritability of 0.17 for SCS and Koeck et al. (2010) estimated a heritability of 0.13 for SCS (based on the arithmetic mean of all milk samples from different lactations). Our heritability estimate of 0.16 ± 0.08 is in line with these results. Yin et al. (2012) showed that the heritability of SCS varies in a wide range over the lactation with peak values near 0.50. Using an average of all milk samples of one lactation in a univariate analysis (Rupp and Boichard, 1999, Koeck et al.,

2010) yields slightly higher heritabilities than the use of one or two milk samples in a univariate analysis (Heringstad et al., 2006 and our work). For mean hyperkeratosis we estimated a heritability of 0.22 ± 0.06, which is in line with Seykora and McDaniel (1985) who estimated a heritability of 0.24 for this trait.

Genetic parameters for variance of milk contents

The variance of milk content traits/hyperkeratosis score between the udder quarters of a cow was generally a trait with a low heritability ($h^2 < 0.10$). Variance of fat content ($h^2 = 0.06 \pm 0.06$), protein content ($h^2 = 0.01 \pm 0.05$), and urea content ($h^2 = 0.04 \pm 0.06$) showed no significant heritability (SE higher than mean). Also the variances of lactose content ($h^2 = 0.08 \pm 0.06$) and hyperkeratosis ($h^2 = 0.04 \pm 0.03$) were hardly different from zero. In contrast to the small, or practically zero, heritabilities for the variance traits, some of these traits showed high repeatabilities. For example the practically not heritable trait variance of protein content has a repeatability of 0.53 ± 0.05 and variance of lactose content with a heritability of 0.08 ± 0.06 has a repeatability of 0.33 ± 0.05 (Table 4).

From our results we conclude that the variance of none of the milk content traits does appear to be a very useful indicator to reflect the physiological stability of milk production or composition that could be used for genetic improvement. However, the substantial repeatabilities of some of the variability traits suggests that non-genetic events (not only in the present lactation, but possibly also during rearing or in former lactations) may cause a sustained imbalance between milk composition in udder quarters, which may have an effect on the long term performance of a cow or on the composition of milk of different quarters. Forsbäck et al. (2010) suggested that if one quarter is infected by bacteria, this leads to a higher protein content and a lower lactose content in milk of the infected quarter, even in the case of a subclinical mastitis. As proposed by Forsbäck et al. (2010) the between-quarter phenotypic variance of lactose and protein thus may be used as a management tool for early detection of mastitis.

Table 4: Additive genetic variance (σ_a^2), variance of permanent environment (σ_{pe}^2) residual variance (σ_e^2), heritability (h^2), repeatability (w^2), with their standard error (SE), mean accuracy of EBVs for all 4,208 animals in the dataset (r_{TI} all), for phenotyped cows (r_{TI} Cows) and for 30 bulls with at least 10 phenotyped daughters in the dataset (r_{TI} Bulls) for individual udder quarter traits as well as genetic parameters and accuracies for mean and variance of within udder milk content traits. Genetic parameters for mean and variance and for urea content are from univariate analyses, genetic parameters for all other traits are from multivariate analyses.

Trait	$\sigma_a^2 \pm$ SE	$\sigma_{pe}^2 \pm$ SE	$\sigma_e^2 \pm$ SE	$h^2 \pm$ SE	$w^2 \pm$ SE	r_{TI} all	r_{TI} Cows	r_{TI} Bulls
Fat content								
Front Left	0.32 ± 0.21	0.62 ± 0.26	2.65 ± 0.22	0.09 ± 0.06	0.26 ± 0.06	0.33	0.42	0.59
Front Right	0.35 ± 0.22		2.75 ± 0.23	0.09 ± 0.06	0.26 ± 0.06	0.32	0.41	0.58
Rear Left	0.40 ± 0.22		2.45 ± 0.22	0.12 ± 0.06	0.29 ± 0.06	0.39	0.52	0.69
Rear Right	0.50 ± 0.24		2.46 ± 0.21	0.14 ± 0.06	0.31 ± 0.06	0.39	0.51	0.68
Mean	0.29 ± 0.20	0.59 ± 0.26	2.12 ± 0.20	0.10 ± 0.06	0.29 ± 0.06	0.27	0.37	0.50
Var	0.17 ± 0.20	0.17 ± 0.29	2.74 ± 0.25	0.06 ± 0.06	0.11 ± 0.08	0.22	0.29	0.41
Protein content								
Front Left	0.17 ± 0.05	0.20 ± 0.04	0.19 ± 0.02	0.30 ± 0.08	0.65 ± 0.04	0.48	0.68	0.80
Front Right	0.20 ± 0.05		0.22 ± 0.02	0.33 ± 0.07	0.65 ± 0.04	0.50	0.72	0.83
Rear Left	0.09 ± 0.04		0.16 ± 0.02	0.21 ± 0.09	0.65 ±0.04	0.43	0.60	0.73
Rear Right	0.09 ± 0.04		0.17 ± 0.02	0.20 ± 0.09	0.63 ± 0.04	0.41	0.57	0.71
Mean	0.10 ± 0.05	0.21 ± 0.04	0.16 ± 0.02	0.21 ± 0.10	0.66 ± 0.04	0.35	0.49	0.64
Var	$0.39*10^{-3} \pm 0.003$	0.03 ± 0.00	0.02 ± 0.00	0.01 ± 0.05	0.53 ± 0.05	0.10	0.12	0.17
Lactose content								
Front Left	0.01 ± 0.01	0.04 ± 0.01	0.07 ± 0.01	0.10 ± 0.07	0.46 ± 0.05	0.35	0.44	0.61
Front Right	0.01 ± 0.01		0.09 ± 0.01	0.05 ± 0.06	0.37 ± 0.05	0.37	0.89	0.64
Rear Left	0.02 ± 0.01		0.07 ± 0.01	0.16 ± 0.07	0.49 ± 0.05	0.45	0.62	0.77
Rear Right	0.02 ± 0.01		0.30 ± 0.02	0.04 ± 0.03	0.17 ± 0.03	0.41	0.55	0.69
Mean	0.02 ± 0.02	0.01 ± 0.02	0.16 ± 0.04	0.08 ± 0.07	0.44 ± 0.05	0.25	0.33	0.46
Var	0.02 ± 0.01	0.06 ± 0.02	0.16 ± 0.01	0.08 ± 0.06	0.33 ± 0.05	0.25	0.34	0.47

Table 4 (continued): Additive genetic variance (σ_a^2), Variance of permanent environment (σ_{pe}^2) residual variance (σ_e^2), heritability (h^2), repeatability (w^2), with their standard error (SE), mean accuracy of EBVs for all 4,208 animals in the dataset (r_{TI} all), mean accuracy for phenotyped cows (r_{TI} Cows) and mean accuracy for 30 bulls with at least 10 phenotyped daughters in the dataset (r_{TI} Bulls) for individual udder quarter traits as well as genetic parameters and accuracies for mean and variance of within udder milk content traits. Genetic parameters for mean and variance and for urea content are from univariate analyses, genetic parameters for all other traits are from multivariate analyses.

Trait	$\sigma_a^2 \pm$ SE	$\sigma_{pe}^2 \pm$ SE	$\sigma_e^2 \pm$ SE	$h^2 \pm$ SE	$w^2 \pm$ SE	r_{TI} all	r_{TI} Cows	r_{TI} Bulls
Urea content								
Front Left	7.87 ± 3.88	13.10 ± 4.59	30.47 ± 2.96	0.15 ± 0.07	0.41 ± 0.06	0.32	0.44	0.61
Front Right	11.12 ± 4.35	8.10 ± 4.69	32.16 ± 3.07	0.22 ± 0.08	0.37 ± 0.06	0.36	0.50	0.67
Rear Left	6.48 ± 3.75	11.77 ± 4.65	32.40 ± 3.23	0.13 ± 0.07	0.36 ± 0.06	0.30	0.41	0.58
Rear Right	8.89 ± 4.05	7.64 ± 4.69	34.16 ± 3.30	0.18 ± 0.08	0.33 ± 0.07	0.33	0.46	0.63
Mean	9.15 ± 3.96	8.71 ± 4.44	31.29 ± 2.96	0.19 ± 0.08	0.36 ± 0.06	0.34	0.47	0.62
Var	4.67 ± 8.37	25.53 ± 12.34	118.04 ± 10.28	0.04 ± 0.06	0.20 ± 0.07	0.18	0.23	0.33
SCS								
Front Left	1.03 ± 0.24	0.57 ± 0.18	1.63 ± 0.15	0.32 ± 0.07	0.49 ± 0.05	0.47	0.66	0.80
Front Right	0.92 ± 0.25		2.16 ± 0.18	0.25 ± 0.06	0.41 ± 0.05	0.45	0.63	0.78
Rear Left	0.73 ± 0.22		1.74 ± 0.15	0.23 ± 0.07	0.43 ± 0.05	0.44	0.62	0.76
Rear Right	0.59 ± 0.21		2.14 ± 0.17	0.18 ± 0.06	0.35 ± 0.05	0.40	0.55	0.71
Mean	0.34 ± 0.17	0.51 ± 0.18	1.21 ± 0.12	0.16 ± 0.08	0.41 ± 0.06	0.33	0.45	0.60
Var	0.41 ± 0.43	0.44 ± 0.51	5.43 ± 0.40	0.07 ± 0.07	0.14 ± 0.06	0.24	0.32	0.45
Hyperkeratosis								
Front Left	0.11 ± 0.02	0.09 ± 0.02	0.28 ± 0.01	0.24 ± 0.05	0.42 ± 0.03	0.48	0.63	0.81
Front Right	0.13 ± 0.03		0.26 ± 0.01	0.26 ± 0.05	0.45 ± 0.03	0.49	0.64	0.82
Rear Left	0.05 ± 0.02		0.31 ± 0.01	0.12 ± 0.04	0.32 ± 0.03	0.40	0.54	0.72
Rear Right	0.07 ± 0.02		0.27 ± 0.01	0.16 ± 0.05	0.37 ±0.03	0.41	0.53	0.73
Mean	0.07 ± 0.02	0.09 ± 0.02	0.14 ± 0.01	0.22 ± 0.06	0.53 ± 0.03	0.42	0.55	0.71
Var	$0.34*10^{-2}$ ± 0.002	0.00 ± 0.00	0.07 ± 0.00	0.04 ± 0.03	0.09 ± 0.04	0.25	0.32	0.47

Genetic parameters on the udder quarter level

The results from multivariate analysis of udder quarter milk content traits are shown in Table 4. Except for hyperkeratosis the heritabilities from multivariate analysis were on average higher than the heritabilities from univariate analysis of the mean content trait.

A multivariate analysis for milk contents of different udder quarters also had advantages for the accuracy of EBVs. A multivariate analysis of milk composition traits (Table 4) led to r_{TI} of EBVs for each udder quarter that were on average higher than the r_{TI} of EBVs estimated for the mean content traits (Table 4). The advantage of multivariate analysis for r_{TI} seems to be higher than the advantage on heritability and is also observed for hyperkeratosis. It was only the r_{TI} of EBVs for urea content which did not benefit, because r_{TI} was derived from univariate analyses since the multivariate analyses of individual udder quarter urea content did not converge.

As for the analysis of milk content on the phenotypic scale, some regularities could also be observed for variance components. Protein content, SCS and hyperkeratosis showed higher heritabilities on the front quarters, fat content had a higher heritability for the rear udder quarters. No clear difference could be observed for lactose content and urea content. For fat content, SCS, and hyperkeratosis the higher heritability for the front/rear quarters is due to both, higher additive genetic variance and lower residual variance. In general, the influence of higher additive genetic variance is more distinct than the influence of lower residual variance. For protein content the heritability for the front quarters is higher, even though the residual variance of the front quarters is higher as well.

In Figure 1 - 3 phenotypic, additive genetic and residual correlations between the udder quarters for the different traits are shown. The additive genetic correlation and the phenotypic correlation of urea content (Figure 2) for the four udder quarters was close to 1 with small SD in all cases (0.93 ± 0.18 to 0.99 ± 0.11 for the additive genetic correlation, 0.90 ± 0.08 to 0.94 ± 0.06 for the phenotypic correlation). The residual correlations between udder quarters for urea content were slightly lower (0.86 ± 0.12 to 0.91 ± 0.10). In contrast to this, the additive genetic and phenotypic correlations of fat content, protein content and lactose content (Figure 1 and 2) were on average weaker and had higher SD (i.e. 0.62 ± 0.47 to 0.99 ± 0.19 for additive genetic correlation of fat content). Remarkably, the correlations between the two front and the two rear quarters were always higher than the correlation between a front and a rear quarter, respectively. Accordingly for fat content we found additive genetic correlations between the two rear quarters of 0.99 ± 0.19 and 0.76 ± 0.42 between the two front quarters but additive genetic correlations of just

0.62 ± 0.47 between the left quarters and 0.64 ± 0.44 between the right quarters. The lowest additive genetic (0.11 ± 0.50 to 0.57 ± 0.34) and phenotypic correlations (0.43 ± 0.16 to 0.55 ± 0.15) were found for SCS between quarters (Figure 3).

This might reflect the different ways milk contents are synthesized. Milk fat is synthesized by different enzymes on the rough endoplasmatic reticulum (Baumann et al., 2006), proteins are produced enzymatically on the surface of the ribosomes and are enzymatically modified in the golgi apparatus (Schmidt, 1971). Lactose is also enzymatically synthesized from glucose (von Engelhardt and Breves, 2004). In contrast to these milk contents, urea is not synthesized in the secretory cells of the udder, but in the liver in order to metabolize the toxic ammonia. From there it is transported via the blood into the udder and then secreted into the milk. Milk is just a second pathway for excreting urea besides urine (von Engelhardt and Breves, 2004). This is supported by findings of Nielsen et al. (2005), who compared milk contents between healthy udder quarters and udder quarters suffering from mastitis. The authors found that urea content does not differ between healthy and affected quarters, while affected quarters show significantly higher protein contents and significantly lower lactose contents. Thus fat, protein and lactose are produced in each quarter individually, while urea is not. These by trend different heritabilities between front and rear quarters and the weaker additive genetic correlations between front and rear quarters (in contrast to the front quarters or to the rear quarters) show that fat content, protein content and lactose content of front and rear quarters could partly be considered as different traits. Due to the high SE of correlations between front and rear quarters this suggestion is very obvious for lactose content (Figure 2).

If records for single quarters were available from routine testing or with increased use of automatic milking systems, the prime benefit would certainly be in the use of this information for the purpose of monitoring and managing animal health (e.g. detecting single quarters that deviate in production or milk composition from the other quarters, which may be an early indicator for mastitis). However, aiming at a more balanced milk production or composition across quarters or giving a higher weight to more informative udder quarters in an index may make sense from a breeding point of view. Larger studies than the presented pilot study are needed to assess whether this is a path that is worth pursuing.

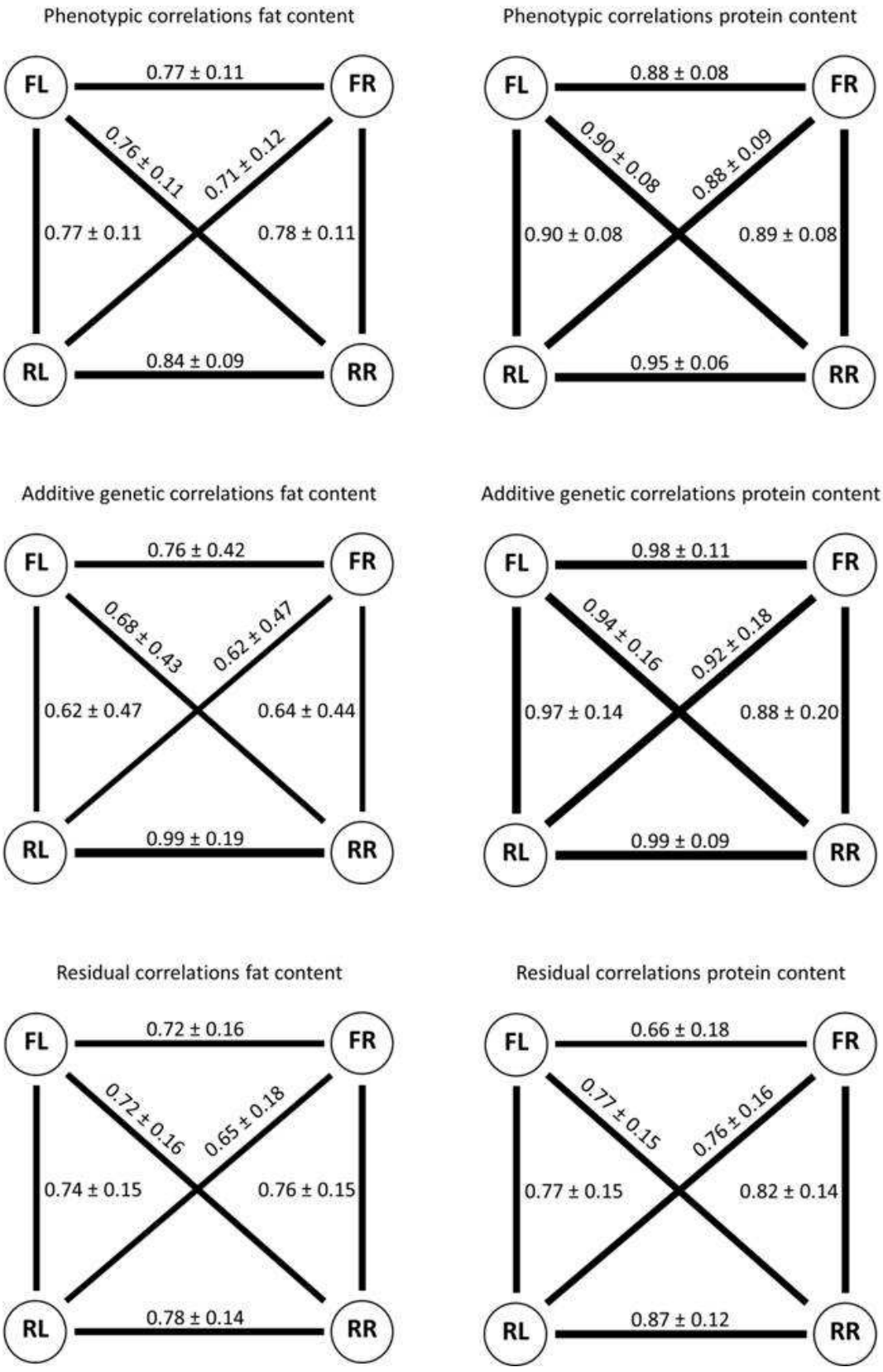

Figure 1: Phenotypic, additive genetic and residual correlations between front left (FL), front right (FR), rear left (RL) and rear right (RR) udder quarter for fat content and protein content. Line thickness is proportional to the correlations between the udder quarters.

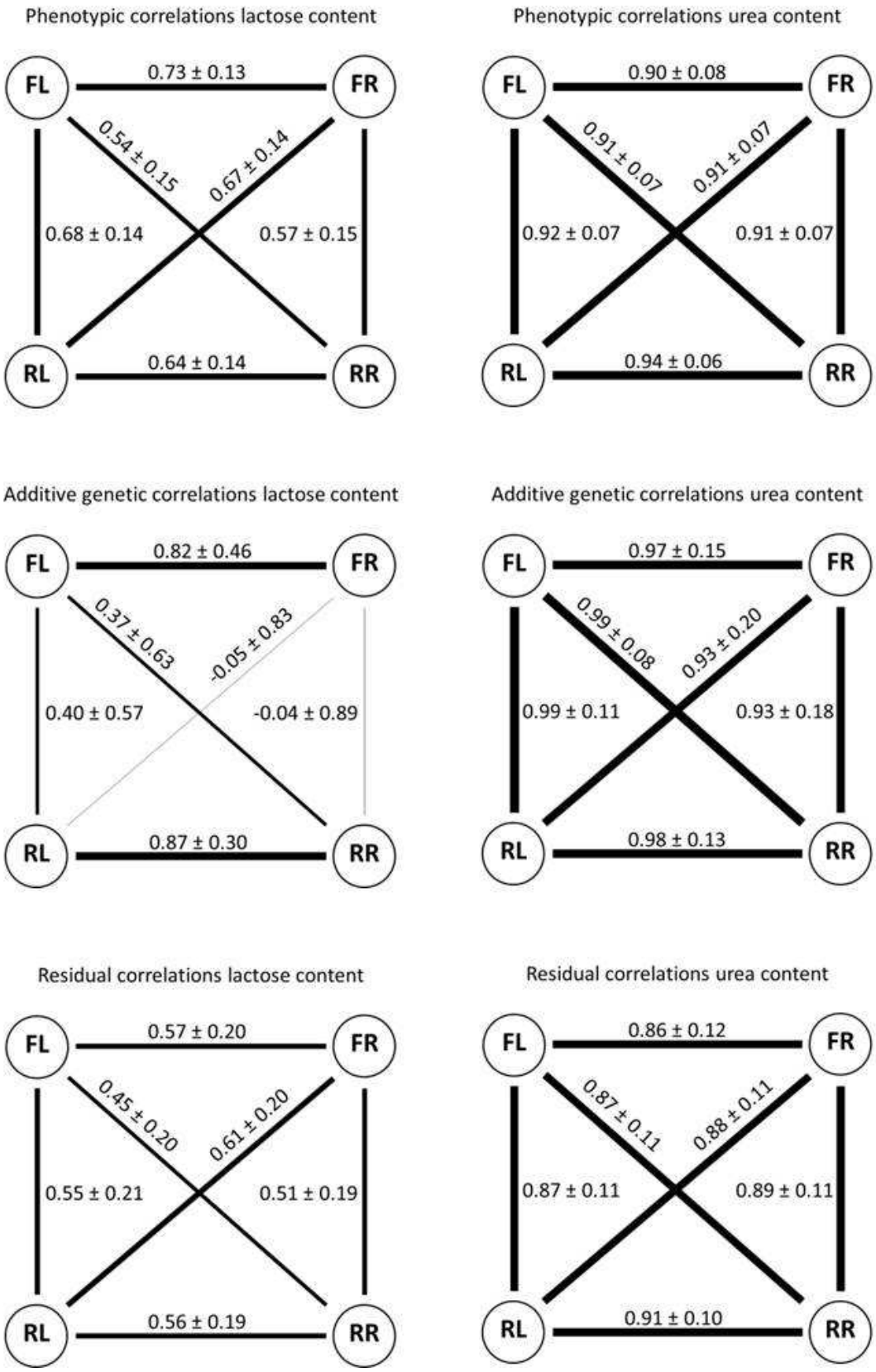

Figure 2: Phenotypic, additive genetic and residual correlations between front left (FL), front right (FR), rear left (RL) and rear right (RR) udder quarter for lactose content and urea content. Line thickness is proportional to the correlations between the udder quarters.

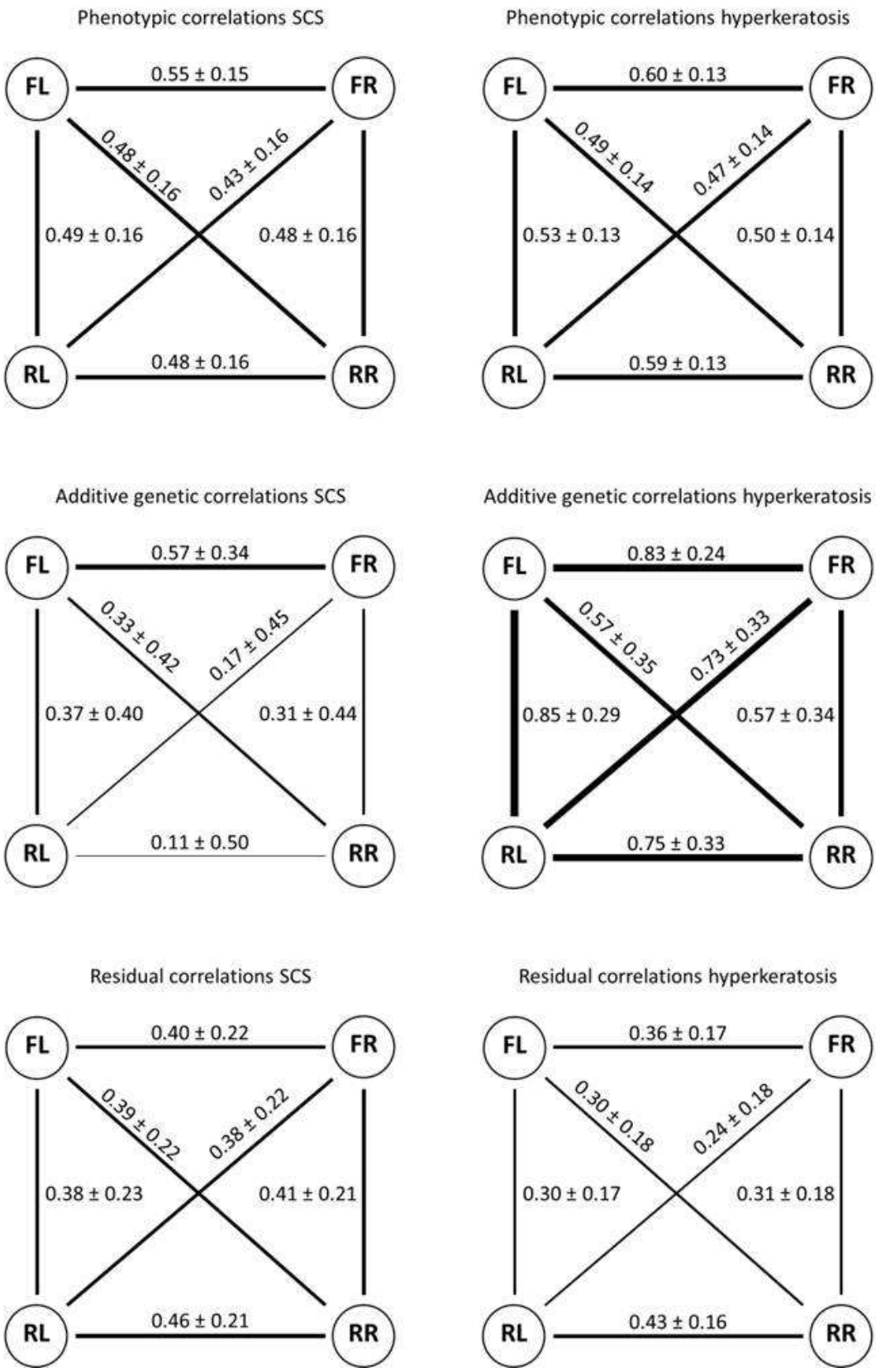

Figure 3: Phenotypic, additive genetic and residual correlations between front left (FL), front right (FR), rear left (RL) and rear right (RR) udder quarter for somatic cell score (SCS) and hyperkeratosis. Line thickness is proportional to the correlations between the udder quarters.

CONCLUSIONS

We showed that recording traits for the udder quarters separately allows a more differentiated assessment of milk composition and udder health, which can be used for management and breeding purposes and as indicators of udder health. There were significant systematic differences in content of fat, protein and lactose between front and rear udder quarters, while content of urea, SCS, and hyperkeratosis did not systematically differ between front and rear quarters. We also found systematic differences in heritabilities for fat content, protein content, and hyperkeratosis but not for urea content and lactose content. This is due to the different tissues where the milk constituents are synthetized. The variance of milk content trait is of limited informational value as an auxiliary trait to breed for udder health, but nevertheless may be a helpful indicator trait to detect beginning or subclinical mastitis.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge co-funding from the European Commission, under the Seventh Framework Program for Research and Technological Development, for the Collaborative Project LowInputBreeds (Grant agreement No 222623). However, the views expressed by the authors do not necessarily reflect the views of the European Commission, nor do they in any way anticipate the Commission's future policy in this area.

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4th Chapter

Accuracy of Direct Genomic Values for Functional Traits in Brown Swiss Cattle

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Published in:
Journal of Dairy Science 97: 1774 - 1781

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ABSTRACT

In this study direct genomic values for the functional traits general temperament, milking temperament, aggressiveness, rank order in herd, milking speed, udder depth, position of labia, and days to first heat in Brown Swiss dairy cattle were estimated based on 777k SNP information from 1,126 animals. Accuracy of direct genomic values was assessed by a fivefold cross-validation with 10 replicates. Correlations between de-regressed proofs and direct genomic values were 0.63 for general temperament, 0.73 for milking temperament, 0.69 for aggressiveness, 0.65 for rank order in herd, 0.69 for milking speed, 0.71 for udder depth, 0.66 for position of labia, and 0.74 for days to first heat. Using the information of the 54k SNP only led to marginal deviations in the observed accuracy. Trying to predict the 20% youngest bulls led to correlations of 0.55, 0.77, 0.73, 0.55, 0.64, 0.59, 0.67, and 0.77 for the traits listed above. Using a novel method to estimate the accuracy of a direct genomic value, defined as correlation between direct genomic value and true breeding value, accounting for the correlation between direct genomic values and conventional breeding values revealed accuracies of 0.37, 0.20, 0.19, 0.27, 0.48, 0.45, 0.36, and 0.12 for the traits listed above. These values are much smaller but probably also more realistic given the heritabilities and samples sizes in this study. Annotation of the largest estimated SNP effects revealed two candidate genes affecting the traits general temperament and days to first heat.

Key words: *direct genomic value prediction, accuracy of direct genomic value, functional trait, gene annotation*

INTRODUCTION

Genomic selection is well established in dairy cattle breeding and accuracies of direct genomic values (**DGV**) for traditional production or conformation traits are high (e.g. Segelke et al., 2012). The benefit of genomic selection should also be utilized for scarcely recorded functional traits which are becoming more and more relevant in dairy cattle breeding. For this reason one aim of our study was to apply the estimation of DGV to a set of functional traits. High density chips with information of more than 777,000 single nucleotide polymorphisms (**SNP**) are becoming increasingly available in cattle breeding. We thus compared results from an estimation of DGV with 777k data with the results of estimation of DGV with 54k data. In order to assess the accuracy of DGV it is easy to calculate the correlation between DGV and conventional breeding value (**EBV**), but this correlation is different from the correlation between DGV and true breeding value (**TBV**) which is normally used in practical breeding to reflect the accuracy of a breeding value. To account for this point the correlation between DGV and EBV is often divided by the accuracy of the EBV. As stated by Amer and Banos (2010), this correction leads to an overestimation of accuracy, so we applied a new method proposed by Wellmann et al. (2013) to estimate more realistic accuracies. To date there are only few genome wide association studies for functional traits in Brown Swiss (**BS**) cattle (see e.g. Guo et al., 2012). Another aim of our work was thus to screen the genome for SNP with large effects on the observed traits in order to find genes that are associated with these SNP and could influence the respective trait.

MATERIAL AND METHODS

Genotypic data

777k genotypes used in this study were derived by imputation from a data set of 880 BS animals genotyped with the Illumina BovineHD chip (727 cows, 153 bulls) and from a dataset of 6,016 animals genotyped with the Illumina Bovine SNP50 chip (548 cows, 5,468 bulls; both chips distributed by Illumina, San Diego, CA). Before imputation both data sets were quality checked separately: SNP with minor allele frequency < 0.5%, a call rate < 90%, missing position, or position on the sex chromosomes were excluded. Within the 777k data set additionally mitochondrial SNP and one SNP of each 55 pairs of SNP with identical position but different denomination, respectively, were excluded. Animals with a call rate < 90% were excluded from both data sets. After quality checks imputation

was done by FImpute (Sargolzaei et al., 2011) from 39,004 SNP to 627,306 SNP using a combination of family and population imputation. This method led to a correlation of 0.99 between true genotype and imputed genotype if both parents were genotyped with the 777k chip and to a correlation of 0.97 between true genotype and imputed genotype if no close relatives were genotyped with the 777k chip (Gredler et al., 2013). We used a subset of 1,126 animals (930 cows from Switzerland with phenotype information for the traits observed and 196 bulls with performance records of at least one daughter) for estimation of DGV and SNP effects in our study.

Phenotypic data

De-regressed proofs (**DRPF**) for the traits general temperament, milking temperament, aggressiveness, rank order in herd, milking speed, udder depth, position of labia, and days to first heat of the 1,126 BS animals were used as quasi phenotypes. For detailed information on the trait definitions see Kramer et al. (2013). Although some of these traits are routinely evaluated and MACE proofs would have been available we decided to use our own breeding values in order to evaluate the possibilities of genomic estimation based on genotypes and phenotypes of a mixed dataset of cows and bulls. The DRPF for individual *i* within a given trait were derived as described in Garrick et al. (2009):

$$DRPF_i = \frac{EBV_i}{r^2_{EBV\,i}}$$

where $DRPF_i$ is the de-regressed proof of individual *i*, EBV_i is the estimated breeding value for individual *i*, and r^2_{EBV} is the squared accuracy of the EBV_i for individual *i*. In our study EBV of cows with several phenotypic measurements as well as EBV of bulls with a different number of daughters (mean: 3.22; range: 1 – 31) were used. Distribution of birth year of animals is shown in Figure 1.

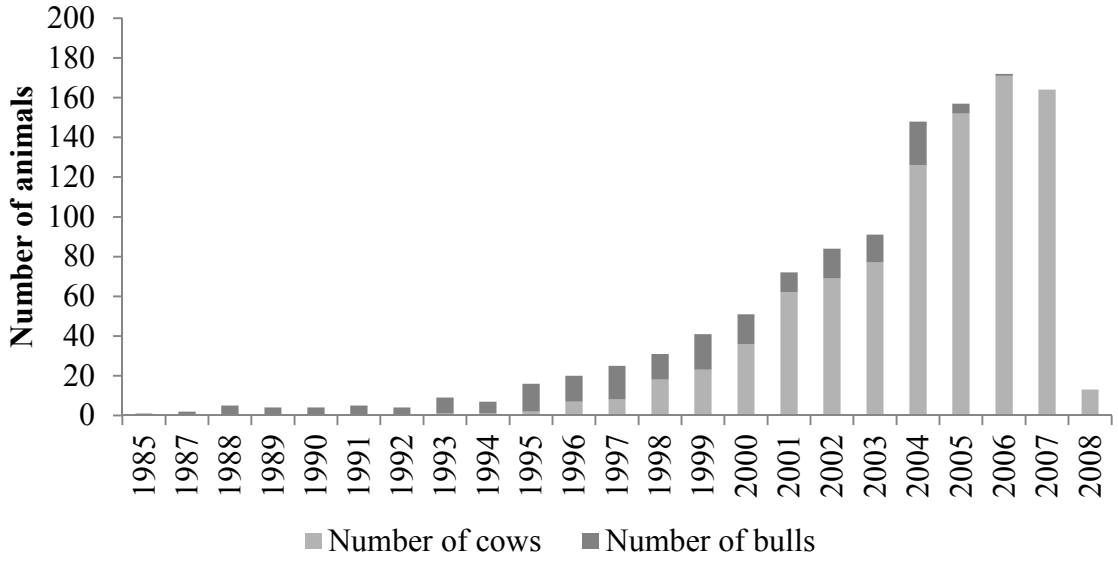

Figure 1: Distribution of birth year for genotyped cows and bulls.

95 per cent of bulls were born between the years 1988 and 2004, while 95 per cent of the cows were born between the years 1998 and 2007. Accuracies of conventional breeding values (r_{EBV}) were in a wide range and on average were higher in cows than in bulls for most traits. (Table 1).

Table 1: Heritabilities (h^2) with standard error (SE), mean accuracies (r_{EBV}) with standard deviation (SD) of estimated/conventional breeding values (EBV) and number of records used for estimation of EBV (n EBV) for the different groups of animals for the traits observed.

Trait	h^2 ± SE	r_{EBV} ± SD all animals (n=1,126)	r_{EBV} ± SD cows (n=930)	r_{EBV} ± SD bulls (n=196)	n EBV
General temperament	0.38 ± 0.07	0.66 ± 0.08	0.68 ± 0.05	0.59 ± 0.12	2,312
Milking temperament	0.04 ± 0.04	0.30 ± 0.06	0.30 ± 0.05	0.29 ± 0.09	2,259
Aggressiveness	0.12 ± 0.08	0.34 ± 0.08	0.34 ± 0.08	0.33 ± 0.10	2,309
Rank order in herd	0.16 ± 0.06	0.51 ± 0.07	0.51 ± 0.05	0.47 ± 0.12	2,304
Milking speed	0.42 ± 0.06	0.72 ± 0.08	0.74 ± 0.05	0.62 ± 0.12	4,540
Udder depth	0.42 ± 0.06	0.66 ± 0.08	0.67 ± 0.06	0.58 ± 0.12	2,195
Position of labia	0.28 ± 0.06	0.61 ± 0.08	0.62 ± 0.07	0.54 ± 0.12	2,232
Days to first heat	0.02 ± 0.04	0.18 ± 0.04	0.19 ± 0.04	0.18 ± 0.06	1,678

Model

DGV were estimated using the model:

$$\mathbf{y} = \mathbf{1}\mu + \mathbf{Zg} + \mathbf{e}$$

where **y** is a vector of DRPF, **1** is a vector of ones, μ is the overall mean, **g** is a vector of additive genetic effects, **e** is a vector of residual effects, and **Z** is a design matrix allocating genetic effects to the observations. For random elements the distributions

$$\mathbf{e} \sim N(0, \mathbf{R}\sigma_e^2)$$

and

$$\mathbf{g} \sim N(0, \mathbf{G}\sigma_g^2)$$

were assumed, where **R** is a diagonal matrix with weighting factors $1/w_i$ on the diagonal and **G** is a genomic relationship matrix. Additive genetic variance (σ_g^2) and residual variance (σ_e^2) were estimated from the complete dataset using ASReml (Gilmour et al., 2009).

Because of the wide range of r_{EBV} also residual errors were in a wide range and it was thus necessary to account for different residual variances. The weighting factor for the reciprocal of the residual error of individual *i,* w_i was calculated as described in Garrick et al. (2009):

$$w = \frac{1 - h^2}{h^2\left(c + \frac{1 - r_{EBV_i}^2}{r_{EBV_i}^2}\right)}$$

where h^2 is the heritability of the observed trait, c is the fraction of genetic variance that is not explained by markers, and $r_{EBV_i}^2$ is the squared accuracy of the EBV. We assumed that the complete genetic variance was explained by the 627k SNP markers and so c was set

to 0. This assumption is also made in routine breeding value estimation for functional traits in Simmental Cattle in Germany and Austria (Edel et al. 2011).

The genomic relationship matrix **G** was set up from the SNP data as described in VanRaden (2008):

$$\mathbf{G} = \frac{(\mathbf{M}-\mathbf{P})(\mathbf{M}-\mathbf{P})'}{2\sum_{j=1}^{m} p_j(1-p_j)}$$

where p_j is the frequency of the second allele at locus j, m is the total number of SNP, **M** is a matrix with genotypes coded as 0, 1, and 2 in columns and animals in rows. **P** is a matrix with all elements in column j being $2p_j$.

The correlation between DGV and DRPF ($r_{DGV,DRPF}$) was assessed by random fivefold cross-validation with 10 replicates and was calculated as an average over all 50 replicates of predicted folds.

To study the influence of the age structure, we also performed one cross-validation run with 777k SNP information with animals sorted by age where DGV of the youngest 177 animals from birth year 2007 – 2008 (approximately 1/5) were predicted with use of the older animals (birth year 1985 - 2006).

Random cross-validation was further carried out with the 54k SNP panel. For cross-validation with 54k information the dataset was restricted to the 39,004 SNP from the 54k SNP chip after filtering and σ_g^2 and σ_e^2 were estimated again using a **G** matrix based on 54k information only.

SNP effects were derived for the 777k SNP data as described in e.g. Ober et al. (2012):

$$\hat{\mathbf{s}} = \frac{\hat{\sigma}_g^2}{2\sum_{j=1}^{m} p_j(1-p_j)}(\mathbf{M}-\mathbf{P})'\left(\hat{\sigma}_g^2\mathbf{G}+\hat{\sigma}_e^2\mathbf{R}\right)^{-1}(\mathbf{y}-\mathbf{1}\hat{\mu})$$

Where $\hat{s}$ is a vector of SNP effects, and all other factors are as described above.

SNP effects were then standardized as follows:

$$\hat{\mathbf{s}}_{\mathbf{STD}} = \frac{\hat{\mathbf{s}}}{\sqrt{\hat{\sigma}^2_{SNP}}}$$

with:

$$\hat{\sigma}^2_{SNP} = \frac{\hat{\sigma}^2_g}{2\sum_{j=1}^{m} p_j\left(1-p_j\right)}$$

Where $\hat{s}_{STD}$ is a vector of standardized SNP effects and all other factors are described above. SNPs with an absolute standardized effect > 4*SD were considered as SNPs with high influence on the specific trait. In the Manhattan plots (not shown) some distinct peaks formed by the high influence SNP were obvious. All genes up to 100 kb upstream and downstream of the SNP position with the most extreme value within these peaks of high influence SNP were annotated using the map viewer option of the bovine genome sequence assembly (*Bos taurus* 6.1; available online at http://www.ncbi.nlm.nih.gov/projects/mapview/map_search.cgi?taxid=9913&build=6.1) in order to find genes that might causatively influence the respective trait.

The correlation $r_{DGV,DRPF}$ is different from the correlation between DGV and TBV, which is the accuracy of genomic breeding values (r_{DGV}). Note that the often reported reliability of genomic breeding values is the square of the accuracy (r^2_{DGV}) used in our study. Often r_{DGV} is estimated as $r_{DGV,DRPF}/r_{EBV}$, where further is assumed that $r_{DGV,DRPF} = r_{DGV,EBV}$ It was noted by Amer and Banos (2010) that with this approach an overlap of testing and training sets in cross-validation may lead to an overestimation of r_{DGV}.

Wellmann et al. (2013) suggested to correct for this bias by fitting a model:

$$\hat{r}_{DGV} = r_{DGV,DRPF} - \hat{a}_1\left(r_{EBV}^{VS} - 1\right) \qquad \text{(eq. 1)}$$

where $\hat{r}_{DGV}$ is the estimated accuracy of DGVs defined as correlation between DGV and TBV, $r_{DGV,DRPF}$ is the estimated correlation between DGV and DRPF in one fold of a cross-validation, r_{EBV}^{VS} is the mean accuracy of EBV in the validation set of this fold, and $\hat{a}_1$ is a regression coefficient estimated from the equation:

$$r_{DGV,DRPF} = a_0 + a_1 r_{EBV}^{VS} + a_2 r_{EBV}^{TS} + e \qquad \text{(eq. 2)}$$

where a_0, a_1, and a_2 are the intercept and fixed regression coefficients, e is the residual error, and r_{EBV}^{TS} is the mean accuracy of EBV in the training set of the specific fold. For further details see Wellmann et al. (2013).

For this approach datasets with different accuracy of conventional breeding values (r_{EBV}) are required to fit the regression model. As described in Wellmann et al. (2013) we performed this by fitting eq. 2 for all traits simultaneously.

If the accuracy of DGV is compared to the accuracy of EBV, EBV should be only based on pedigree information since these are also available in an early stage of an animal's life. We thus approximated accuracies of EBV based on pedigree information from the average accuracies of EBV of bulls and cows (Table 1) by the formula:

$$r = \sqrt{0.25\left(r_S^2 + r_D^2\right)}$$

Where r_S^2 is the reliability of the sire's EBV and r_D^2 is the reliability of the dam's EBV. This formula is also used by e. g. Edel et al. (2010)

RESULTS AND DISCUSSION

Correlation between DGV and DRPF

The values for $r_{DGV,DRPF}$ from five-fold random cross-validation with 10 replicates with 777k information are shown in Figure 2. With random cross-validation we obtained $r_{DGV,DRPF}$ (± SD) of 0.63 ± 0.05 for general temperament, 0.73 ± 0.04 for milking temperament, 0.69 ± 0.06 for aggressiveness, 0.65 ± 0.04 for rank order in herd, 0.69 ± 0.03 for milking speed, 0.71 ± 0.03 for udder depth, 0.66 ± 0.03 for position of labia, and 0.74 ± 0.02 for days to first heat between DGV and DRPF. The correlations between DGV and DRPF after stratification by age were in a range of 0.55 and 0.77 and are thus on average slightly lower than correlations from random cross-validation (Figure 2). $r_{DGV,DRPF}$ values for the youngest animals deviate from the average values from random cross-validation between + 0.04 for milking temperament, and aggressiveness and – 0.12 for udder depth but are mostly in the range of values obtained by random cross-validation.

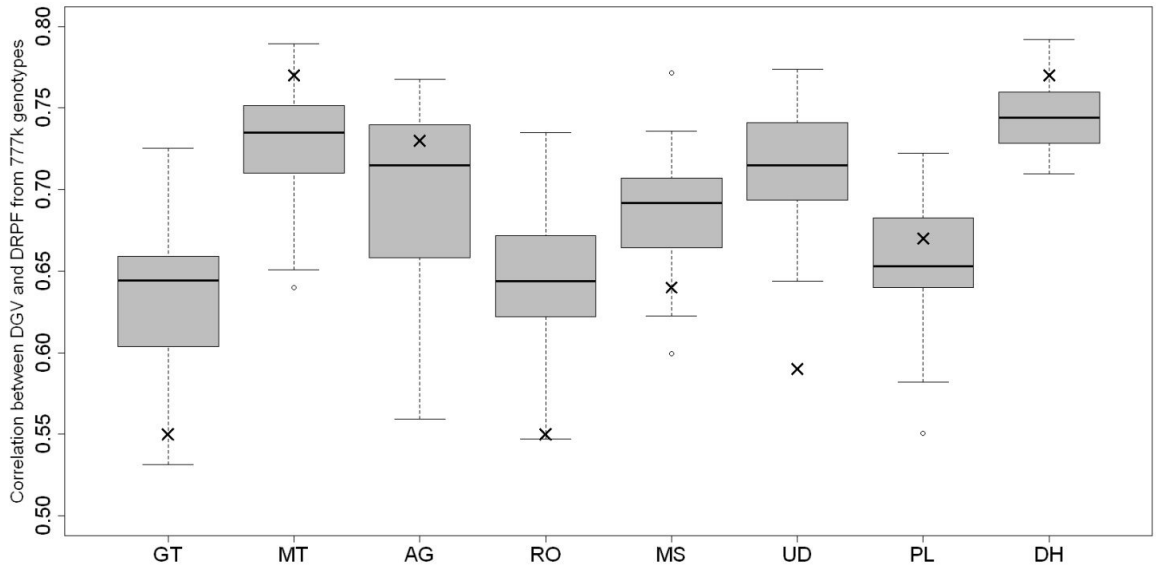

Figure 2: Correlations between direct genomic values (DGV) and de-regressed proofs (DRPF) from cross-validation for general temperament (GT), milking temperament (MT), aggressiveness (AG), rank order in herd (RO), milking speed (MS), udder depth (UD), position of labia (PL), and days to first heat (DH), based on 777k SNP information. Results from 5 fold random cross-validation with 10 replicates are presented by boxes; results from stratification by age are presented by **X**.

These results are in line with Saatchi et al. (2011) who compared random cross-validation and cross-validation by age with 16 traits of American Angus beef cattle and also found higher accuracy for most traits with random cross-validation compared to stratification by age. If dairy cattle breeding values are estimated in practice, information from older animals (EBV of bulls with a certain number of daughters in milk or phenotypic measurements of lactating cows) is used to predict DGV for young animals, e.g. bull calves for purchase by AI organizations. Thus the age-stratified approach reflects the practical relevant case. However, due to the lack of re-sampling the point estimate obtained for $r_{DGV,DRPF}$ in the age stratified scenario may have a large stochastic error and should be interpreted with caution. Erbe et al. (2011) have suggested an age-stratification with re-sampling which provides a distribution of estimates for the accuracy, however this approach could not be implemented here due to the limited sample size.

In general, $r_{DGV,DRPF}$ values in our study are high, especially if the small number of animals in the dataset (n = 1,126) and the low r_{EBV} (0.18 – 0.72) are taken into account. Erbe et al. (2012) found correlations between genomic breeding values and daughter trait deviations of 0.58, 0.58, and 0.56 for the traits milk yield, fat yield, and protein yield respectively in a dataset of 2,257 Australian Holstein bulls. Thus we reached seemingly higher correlations between DGV and DRPF although our dataset was smaller and r_{EBV} was lower. This might be due to overestimation of accuracy of DGV which will be discussed later.

Using 54k genotype information (Figure 3), $r_{DGV,DRPF}$ were in a range between 0.74 ± 0.02 (days to first heat) and 0.63 ± 0.05 (general temperament) and thus show a difference to results from cross-validation with 777k genotype information of less than 0.01 for all traits. This finding is in line with results from Su et al. (2012) with Nordic Holstein and Red Dairy Cattle. The authors found only slight differences in reliability for DGV between 54k genotype data and 777k genotype data of up to 0.03 for the traits protein, fertility, and udder health. Our results are also in line with results from Erbe et al. (2012) who found correlations between genomic breeding value and daughter trait deviation from 54k data being 0.01 to 0.03 less than those obtained with 777k data in Australian Holstein Cattle.

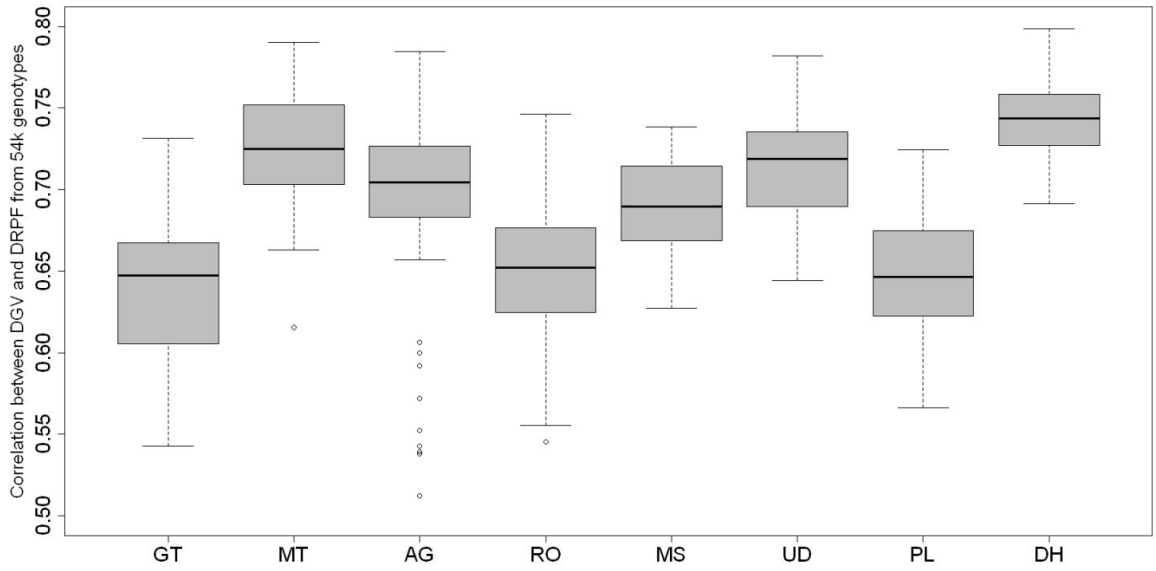

Figure 3: Correlations between direct genomic values (DGV) and de-regressed proofs (DRPF) from cross-validation for general temperament (GT), milking temperament (MT), aggressiveness (AG), rank order in herd (RO), milking speed (MS), udder depth (UD), position of labia (PL), and days to first heat (DH), based on 54k SNP information.

This indicates that marker density of the 54k SNP chip appears sufficient and using the more expensive 777k SNP chip does hardly provide any improvement for predictive ability. This finding is further supported by e.g. VanRaden et al. (2013) who found that the HD SNP chip only provides slightly better results. With use of a HD SNP chip the authors estimated a reliability of 61.1%, with use of a 54k SNP chip they estimated a reliability of 60.7% both as an average of 28 different traits.

Accuracy of DGV

The frequently used approach for calculating the accuracy of DGV as $r_{DGV,DRPF}/r_{EBV}$ would have resulted in accuracies > 1.0 for most of the traits in our dataset that is characterized by high values of $r_{DGV,DRPF}$ and low but highly variable values for r_{EBV} (Table 2). This is a clear case of overestimation of accuracy as described by Amer and Banos (2010). We thus applied the approach of Wellmann et al. (2013) for estimation of r_{DGV}. Values for r_{DGV} for the traits observed are shown in Table 2. The results indicate that it is generally possible to implement a system of genomic breeding value estimation for functional traits based on genotypes of phenotyped cows and sires with phenotyped

daughters. Unfortunately, the limited number of individuals in our study was not sufficient to achieve a large advantage of genomic prediction over parent average (r_{PA}). Further work is needed to evaluate how many individuals have to be genotyped and phenotyped to achieve higher accuracy of GBV.

Table 2: Correlation between direct genomic value and deregressed proof ($r_{DGV,DRPF}$), heritability (h^2), size of the training set (n EBV), accuracy of conventional breeding values from parent average (r_{PA}), accuracy of genomic breeding values following the common approach ($r_{DGV,DRPF}/r_{EBV}$), and accuracy of direct genomic values (r_{DGV}) from application of the formula by Wellmann et al. (2013) for the traits observed.

Trait	$r_{DGV,DRPF}$	h^2	n EBV	r_{PA}	$r_{DGV,DRPF}/r_{EBV}$	r_{DGV}
General temperament	0.63	0.38	2,312	0.45	0.95	0.37
Milking temperament	0.73	0.04	2,259	0.21	> 1	0.20
Aggressiveness	0.69	0.12	2,309	0.24	> 1	0.19
Rank order in herd	0.65	0.16	2,304	0.35	> 1	0.27
Milking speed	0.69	0.42	4,540	0.48	0.96	0.48
Udder depth	0.71	0.42	2,195	0.44	> 1	0.45
Position of labia	0.66	0.28	2,232	0.41	> 1	0.36
Days to first heat	0.74	0.02	1,678	0.13	> 1	0.12

Our results suggest that r_{DGV} as well as r_{PA} are functions of heritability and size of the training set for the respective trait. Milking speed has the highest heritability and the largest size of the training set and also the highest r_{DGV} and r_{PA} were obtained for this trait (Table 2). In contrast, days to first heat has the smallest r_{DGV}, the smallest r_{PA}, the smallest size of the training set and also the smallest heritability (Table 2). In general, computing the observed accuracy of PA can be very helpful, however most cross-validation studies do not test the PA predictions directly, whereas validations using time truncation do.

As can be seen in Figure 4, $r_{DGV,DRPF}$ varies strongly within trait while mean r_{EBV} in the validation sets is trait-specifically quite consistent (from $r_{EBV} \approx 0.20$ for days to first heat to $r_{EBV} \approx 0.70$ for milking speed) across the samples. Fitting eq. 2 to these data resulted in $a_0 = 0.75 \pm 0.01$; $a_1 = -0.76 \pm 0.46$, and $a_2 = 0.64 \pm 0.46$. Despite the large standard errors of the regression coefficients, our results are in line with the general finding of Wellman et al. (2013) in another species (pigs), namely that a_2 is positive and a_1 is negative.

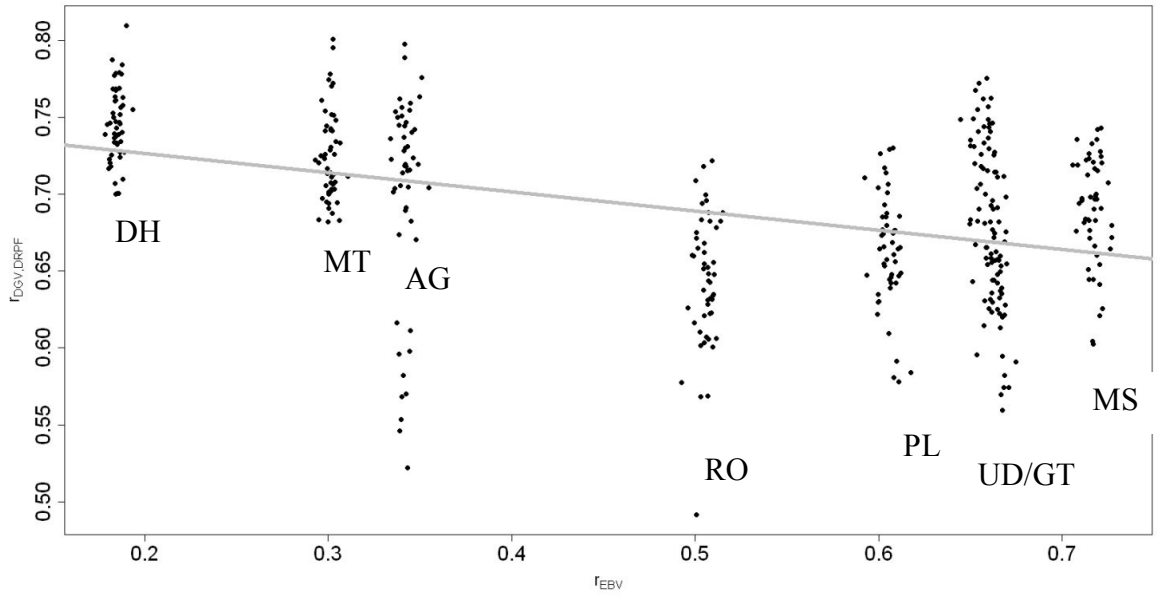

Figure 4: Linear regression of the correlation between direct genomic value and de-regressed proof ($r_{DGV,DRPF}$) on the accuracy of estimated breeding value in the validation set (r_{EBV}), simultaneously for all eight traits days to first heat (DH), milking temperament (MT), aggressiveness (AG), rank order in herd (RO), position of labia (PL), udder depth (UD), general temperament (GT), and milking speed (MS).

This latter result suggests that the accuracy of genomic breeding values estimated as $r_{DGV,DRPF} / r_{EBV}$ is the more biased upward the smaller the accuracy of conventional breeding values r_{EBV} is for a given trait. By shifting the estimates of accuracy from > 0.9 to 0.12 (for days to first heat) to 0.48 (for milking speed) the applied correction provides certainly more realistic estimates of the realized accuracy (Table 2).

As mentioned above standard errors of the estimates for a_0, a_1, and a_2 are high. One way to prevent this high standard errors might be to split the dataset in a non random way into training and validation set, e. g. cross validation by age. This might be an option for larger data sets (e. g. Erbe et al., 2011) but in our limited dataset only one replicate would be possible. Estimates from a regression based on these values will also have large standard errors. This strategy of splitting the data has thus no advantages in our data. We further tried to reduce the standard error by including random effects for trait and cross validation fold in equation 2 to estimate a_0, a_1, and a_2. This led to the same values for a_0, a_1, and a_2 and additionally to a slight increase of the standard error of a_0 as well as a slight decreases of the standard errors for a_1 and a_2 ($a_0 = 0.75 \pm 0.03$; $a_1 = -0.76 \pm 0.38$, and $a_2 = 0.64 \pm 0.38$). Thus, the advantage of this second option seems to be limited.

Results for SNP effects

Genomic regions with clusters of SNPs with large effects (> 4 SD above the mean) on the traits observed are listed in Table 3. When annotating these regions we found two candidate genes that might have an influence on the functional traits observed. The TAC1 gene (tachykinin, precursor 1) is located within a peak on chromosome 4 for general temperament. This gene encodes a member of the tachykinin peptide hormone family, which are said to play a role in behavior response (Chiwakata et al., 1991) and might thus also have an influence on the general temperament of an animal. For the trait days to first heat we found a peak in the center of chromosome 21. Four genes are located here, one of which is the CYP11A1 gene encoding the Cholesterol side-chain cleavage enzyme. This enzyme catalyzes the composition of pregnenolone from cholesterol, a steroid hormone involved in fertility (Heo et al., 2011). CYP11A1 might therefore influence the level of estrogen and thus the time span between parturition and occurrence of first heat.

As stated above, to date there is limited work about genome wide association of functional traits in BS, especially for behavior traits or the new conformation trait position of labia we deal with. We actually found no biologically plausible candidate gene that might influence the trait milking temperament, although there was a distinct peak on chromosome 14. Larger studies and more collaboration between genetics and molecular biology are necessary to identify genes affecting the functional traits we have studied.

Table 3: Localization (Chromosome, Position) of regions with possible high influence on the observed traits, denomination of SNP with highest effect within that region (SNP ID) and genes up to 100 kb upstream and downstream (Gene 100k upstream/downstream) of the SNP with the highest effect within that region. Genes in **bold face** could have an impact on the observed trait.

Trait	SNP ID	Chromosome	Position	Gene 100k upstream/downstream
General temperament	BovineHD0400004398	4	14,887,518	**TAC1**
	BovineHD0800030109	8	101,460,591	AKAP2, TXN, TXNDC8
Milking temperament	BovineHD1400015525	14	55,580,399	
Aggressiveness	BovineHD0800017506	8	58,503,427	
	BovineHD2000018921	20	65,702,103	ADCY2
Rank order in herd	BovineHD0100035735	1	126,516,898	SLC9A9
	BovineHD0600001952	6	8,448,579	
	BovineHD1800009080	18	29,383,349	
	BovineHD1900003727	19	14,062,783	ACACA, TADA2A, DUSP14, SYNRG
Milking speed	BovineHD0500031516	5	109,364,642	CACNA1C, IL17RA, CECR5, CECR1
	BovineHD0900004541	9	16,815,501	
	BovineHD1100024011	11	83,456,178	
	BovineHD1200003011	12	10,668,524	OLFM4
	BovineHD1400018332	14	65,573,091	YWHAZ
Udder depth	BovineHD0300020780	3	70,574,840	TNNI3K
	ARSBFGLNGS13749	6	67,877,316	ATP10D, CORIN
Position of labia	BovineHD0800030481	8	102,549,325	ZNF483
	BovineHD2500002490	25	9,358,007	ATF7IP2, EMP2, TEKT5
Days to first heat	BovineHD0400024614	4	88,917,242	WASL, SPAM1
	BovineHD1500021240	15	73,554,550	
	BovineHD4100015166	21	34,675,608	UBL7, SEMA7A, **CYP11A1**, CCDC33

ACKNOWLEDGEMENTS

The authors gratefully acknowledge co-funding from the European Commission, under the Seventh Framework Program for Research and Technological Development, for the Collaborative Project LowInputBreeds (Grant agreement No 222623). However, the views expressed by the authors do not necessarily reflect the views of the European Commission, nor do they in any way anticipate the Commission's future policy in this area.

Parts of this research were also funded by the German Federal Ministry of Education and Research (BMBF) within the AgroClustEr "Synbreed – Synergistic plant and animal breeding" (FKZ 0315528C).

The authors thank Braunvieh Schweiz, the genotype pool Germany-Austria, Associazione Nazionale Allevatori Bovini della Razza Bruna and Beltsville Agricultural Research Center for provision of genotypes.

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5th Chapter

General Discussion

BREEDING VALUE ESTIMATION FOR NEW TRAITS WITH USE OF GENOMIC SELECTION AND A SYSTEM OF COOPERATOR HERDS

In chapter 4 accuracies of direct genomic values (r_{DGV}) were estimated for the traits general temperament, milking temperament, aggressiveness, rank order in herd, milking speed, udder depth, position of labia, and days to first heat based on the genetic parameters and breeding values estimated in chapter 2. As shown in Table 1 correlations between direct genomic value (**DGV**) and de-regressed proof ($r_{DGV, DRPF}$), used as a first rough assessment for the goodness of the DGV were high (0.63 - 0.74). This was an unexpected result considering the low number of animals for estimation of DGV (n = 1,126) and the low accuracy of estimated breeding values (r_{EBV}) with values between 0.30 and 0.72 (Table 1).

Table 1: Accuracy of estimated breeding value (r_{EBV}), correlation between direct genomic value and de-regressed proof ($r_{DGV,DRPF}$), and accuracy of direct genomic value (r_{DGV}) for the traits general temperament milking temperament, aggressiveness, rank order in herd, milking speed, udder depth, position of labia, and days to first heat.

Trait	r_{EBV}	$r_{DGV, DRPF}$	r_{DGV}
General temperament	0.66	0.63	0.37
Milking temperament	0.30	0.73	0.20
Aggressiveness	0.34	0.69	0.19
Rank order in herd	0.51	0.65	0.27
Milking speed	0.72	0.69	0.48
Udder depth	0.66	0.71	0.45
Position of labia	0.61	0.66	0.36
Days to first heat	0.18	0.74	0.12

The simple correlation $r_{DGV,DRPF}$ neglects r_{EBV} and is also far away from the correlation between an estimated breeding value and the true breeding value, which is usually denoted as accuracy of a breeding value. This value is widely used to describe the goodness of an estimated breeding value in practical dairy cattle breeding. A common method to account for r_{EBV} is to divide $r_{DGV,DRPF}$ by r_{EBV}. As described by Amer and Banos (2010) this method should lead to overestimation of r_{DGV}. In the dataset used in this study, where r_{EBV} were smaller than $r_{DGV,DRPF}$ for some traits (e.g. udder depth and position of labia; Table 1), this approach would have led to reliabilities > 1 and thus is completely

inappropriate for traits where estimated breeding value (**EBV**) can only be estimated with low accuracy (r_{TI}). For this reason, the approach by Wellman et al. (2013) was applied to estimate r_{DGV} for the traits listed above.

As shown in Table 1 this approach delivered realistic r_{DGV} values between 0.12 for days to first heat and 0.48 for milking speed. Regardless of whether these values are realistic, they are far away from accuracies of breeding values a farmer would accept for an artificial insemination sire. König et al. (2009) considered $r_{TI} = 0.80$ as a critical value for acceptance by farmers. Hence, additional information is required to reach r_{TI} values > 0.80 for the functional traits described in this study. König and Swalve (2009) proposed to collect additional phenotypic data of daughters of bulls in cooperator herds. These sources of information have to be combined into an index in order to increase r_{TI} . In this case the resulting breeding value is no longer a DGV but a combined breeding value (**GEBV**) with information from genomic evaluation and conventional evaluation (see chapter 1). Depending on heritability and r_{DGV} , König and Swalve (2009) calculated how many daughters of a bull have to be phenotyped to reach a reliability of the combined breeding value (r_{GEBV}) of at least 0.80. Their results show that it will be problematic to estimate combined breeding values of sufficient accuracy for most of the traits described in this study, due to the low accuracy of DGV. König and Swalve (2009) presume a r_{DGV} of at least 0.5. Due to the limited number of animals in the training set (chapter 4) this value is approximately reached for the traits milking speed and udder depth only. Referring to König and Swalve (2009) 12 – 13 additional phenotyped cows are required to reach a r_{GEBV} of 0.80 for a sire's GEBV for milking speed and udder depth with heritabilities between 0.40 and 0.45 and r_{DGV} close to 0.50.

Given that an average breeding organization aims to test 50 young bulls per year (Gernand et al., 2007), this breeding organization has to ensure a test capacity of 650 first lactation cows by cooperator herds to estimate GEBV of young bulls with r_{GEBV} of at least 0.80 for the traits milking speed and udder depth (50 bulls * 13 phenotyped daughters). In this case, cows of higher lactation in cooperator herds have to be neglected, because they cannot contribute to the r_{GEBV} of their sire at this early stage of a bull's live. In this instance, the generation interval would become too long, and the most important advantage of genomic estimation, the drastic reduction of generation interval (e.g. Schaeffer, 2006), would be

totally lost. Nevertheless, cows of higher lactation in cooperator herds should be genotyped and phenotyped, too. They will contribute to the overall r_{EBV} (see chapter 2), and thus SNP effects can be estimated with higher accuracy and r_{DGV} will slightly increase. Further studies should be done to evaluate how many genotypes are needed to increase r_{DGV} of general temperament, milking temperament, aggressiveness, rank order in herd, position of labia, and days to first heat to a value of at least 0.50.

Taking German Holstein as an example, it can be assumed that on average 35% of cows in the herdbook population are in first lactation (Vit, 2012). Thus cooperator herds with 1,857 cows in total are required to have 650 first lactating cows. With an average herd size in western Germany of 61.22 cows/herd (Vit, 2012), only 30 herds are required as cooperator herds (0.24% of all dairy herds in western Germany), if a breeding organization aims to test 50 young sires per year. In eastern Germany, with an average herd size of 261.32 cows/herd (Vit, 2012), only 7 herds are required as cooperator herds (0.25% of all dairy herds in eastern Germany) to test 50 young sires. These considerations show that it should be possible to estimate GEBV for the functional traits milking speed and udder depth as introduced in chapter 2 within a system of genomic estimation and cooperator herds. The only condition for use of this system is the phenotypes are assessed as accurately as done in chapter 2 of this study.

If breeding organizations aim to display GEBV of r_{TI} > 0.95, for the traits milking speed and udder depth a cohort of 81 daughters per bull have to be additionally phenotyped (König and Swalve, 2009). This number of additional phenotyped daughters could also lead to acceptable r_{GEBV} for further functional traits mentioned in this study. Thus, 4,050 first lactating cows are required to test 50 bulls. Given that 35% of cows are in first lactation (Vit, 2012) this will result in 11,571 cows from all lactations in cooperator herds. To achieve this number of cows by using average sized herds, 189 farms (1.50% of all herds) are required in Western Germany and 44 herds of average size are required in Eastern Germany (1.55% of all herds). This is still only a small proportion of herds, but it will cause considerable logistical efforts to collect genotypic and phenotypic data from 189 different herds. Therefore, the generation of GEBV of this high reliability from herds in Western Germany remains questionable. At this point the logistical advantages of large cooperator herds mentioned by Schierenbeck et al. (2011) and Swalve and König (2007) become obvious.

As described by König and Swalve (2009) it is still necessary to phenotype a small number of daughters to achieve GEBV of acceptable reliability. Thus, the main advantage of a drastically reduced generation interval by genomic estimation (Schaeffer, 2006) is partly lost. However, it is questionable if it would be possible at all to estimate valid EBV for the traits milking speed and udder depth, where phenotyping is cost and time consuming, without a system of genomic evaluation and cooperator herds. In a cooperator herd it is justifiable to install technical devices in order to measure e.g. milking speed, to employ people to assess behavior of cows, or to pay farmers for accurate data recording. Results of Fiedler et al. (2004) show that it is often difficult to generate phenotypic data of sufficient reliability for traits that are scored on a subjective scale, like e.g. calving ease from field data. Thus, it might not be possible to estimate breeding values for traits which cannot be readily measured, if farmers have no direct financial advantage in an assiduous scoring.

To establish a breeding value estimation for milking speed and udder depth as described in chapter 2, it will be necessary to phenotype and genotype an initial sample of 1,000 cows, where SNP effects can be derived in a first step (as described by Schaeffer, 2006). This will generate EBV and DGV of similar reliability as in chapter 4. Furthermore, this is the most labor and cost intensive part of the breeding value estimation. In a second step, DGV of young bulls can be estimated from this information and bulls might be preselected based on their DGV which could slightly decrease the number of cows in cooperator herds. In a third step, cows in cooperator herds are mated to these young bulls in a way that at least 13 daughters are born. Daughters of bulls have to be reared and phenotypes have to be assessed assiduously in the fourth step. The phenotyped daughters of young bulls should also be genotyped, and can thus also contribute to the pool of animals that are used to estimate SNP effects. In the fifth step, DGV of bulls and daughter information can be combined to a GEBV of the young bull as described by König and Swalve (2009).

Finally, it can be concluded that benefit for functional traits can arise form genomic estimation in cooperator herds. The measurement of functional traits is often labor intensive and costly. Nonetheless, these traits show a moderate to high heritability if phenotypes are assessed assiduously. A measurement of functional traits with high accuracy is difficult to perform from field data, but it is possible to get measurements of high accuracy from cooperator herds. Cows belonging to these herds should also be genotyped to have a pool of animals that can be used for estimation of SNP effects. The r_{DGV} out of these cooperator herds is too low for farmers to be accepted as a breeding

value of an AI sire. In order to reach sufficient r_{DGV} for AI sires, a manageable number of additional daughters of young bulls have to be phenotyped. These sires will be used in the whole population. When daughters of young bulls are phenotyped, the reduction of generation interval as a main advantage of genomic estimation is partly lost, but it is still an advantage that estimation of breeding values for functional traits gets possible at all. Breeding organizations have to decide very carefully if there is a market for AI sires with breeding values for functional traits, and if the return of investment is large enough to justify the extra effort for assessing these traits in a system of cooperator herds and genomic estimation. If cooperator herds were not only used to assess the functional traits described in this study but also for further functional traits like fertility or claw disorders, or to improve the breeding value estimation for production traits, the return of investment can even be increased.

ANNOTATION OF GENES WITH POSSIBLE INFLUENCE ON NOVEL FUNCTIONAL TRAITS

In Figure 1 – 8 Manhattan plots with 777k SNP information for the traits general temperament, milking temperament, aggressiveness, rank order in herd, milking speed, udder depth, position of labia, and days to first heat are shown. As stated by Guo et al. (2012) to date only little work is done on genome wide association studies (**GWAS**) for Brown Swiss Dairy Cattle (**BS**). Most studies aiming to find genes which explain a large amount of a trait are performed in the more widely spread Holstein breed (e.g. Cole et al., 2011) or deal with production traits of BS (e.g. Maxa et al., 2012). None of these studies actually used 777k SNP information, as done in this work. This makes it difficult to compare the results of the current work with literature.

Manhattan plots for the behavior traits general temperament, milking temperament, aggressiveness, and rank order in herd are shown in Figure 1 – 4. To date there is no work about GWAS of behavior traits in dairy cattle, thus the SNP effects for behavior traits cannot be compared to literature but just among each other.

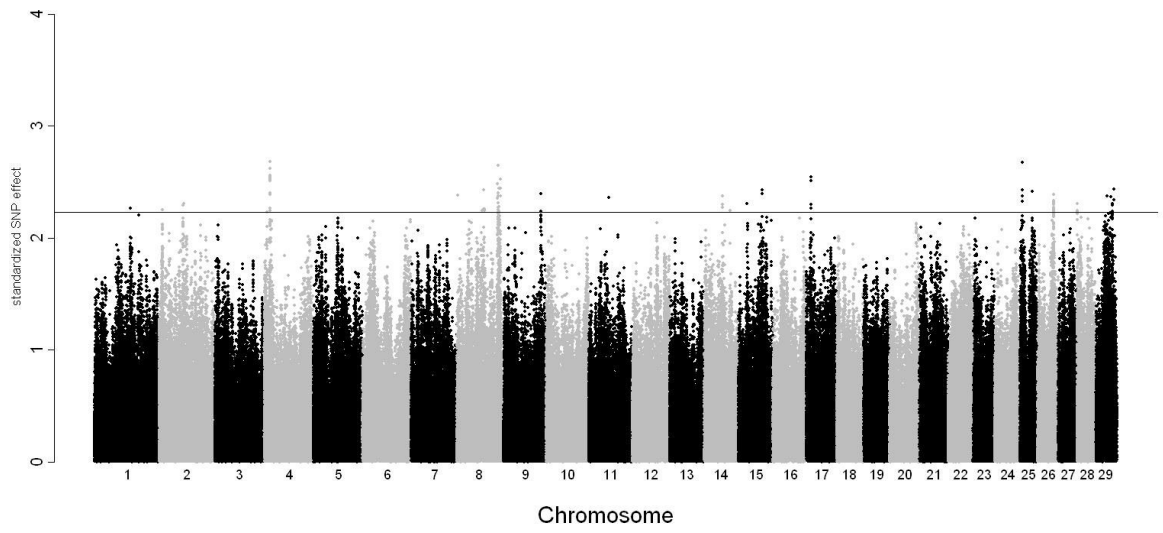

Figure 1: Manhattan plot for the trait general temperament. SNP above the horizontal line have a standardized effect of more than 4*SD.

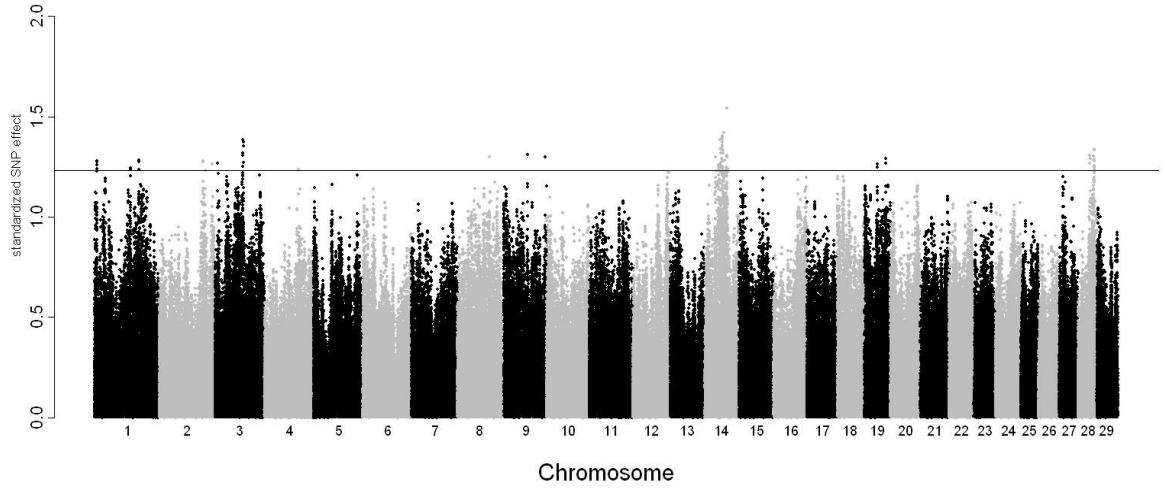

Figure 2: Manhattan plot for the trait milking temperament. SNP above the horizontal line have a standardized effect of more than 4*SD.

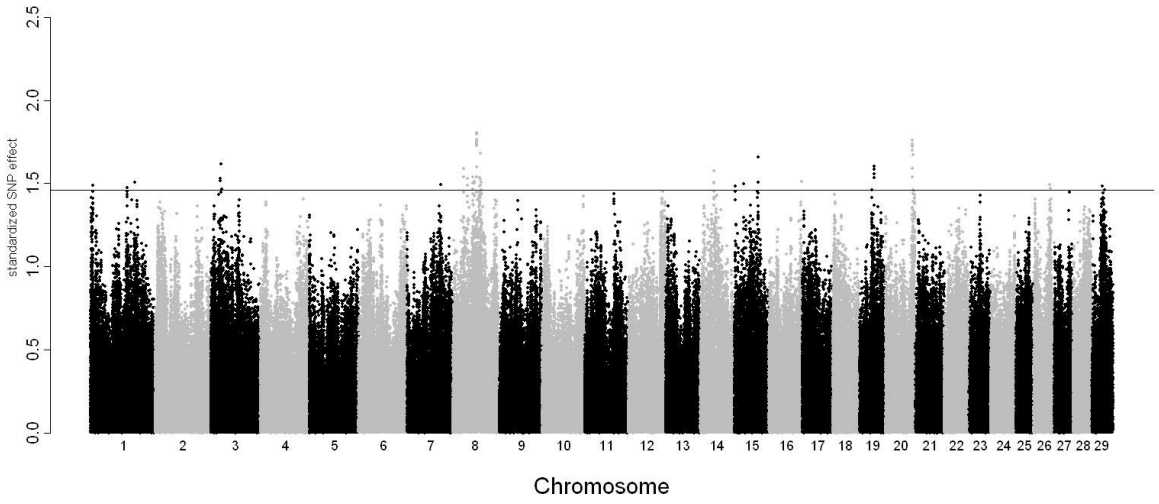

Figure 3: Manhattan plot for the trait aggressiveness. SNP above the horizontal line have a standardized effect of more than 4*SD.

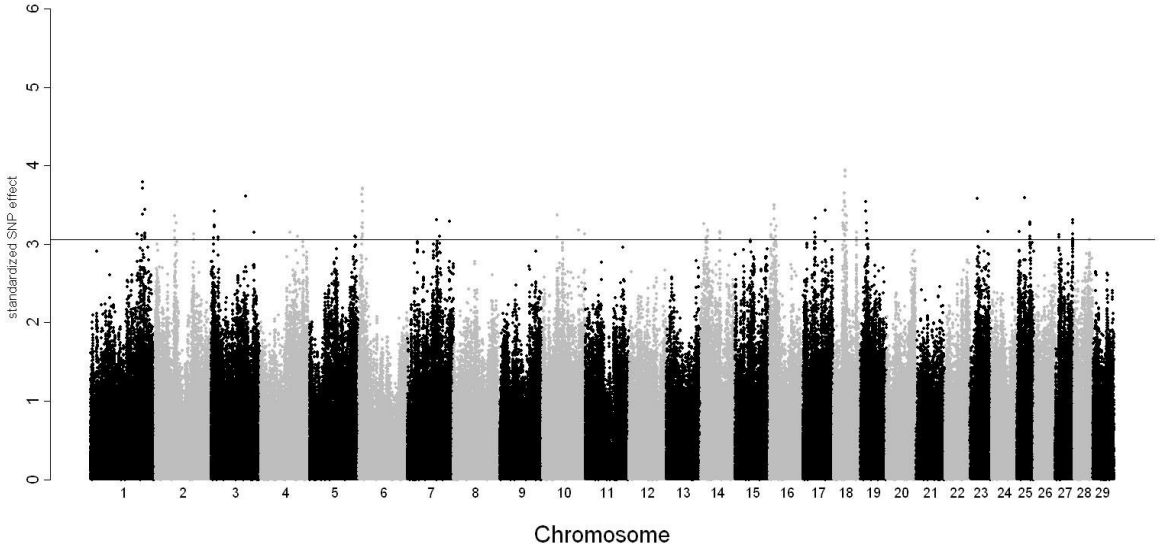

Figure 4: Manhattan plot for the trait rank order in herd. SNP above the horizontal line have a standardized effect of more than 4*SD.

As stated in chapter 2, the traits general temperament, milking temperament, aggressiveness, and rank order in herd belong to the same trait complex (behavior) but have to be considered as different traits. Likewise, this becomes obvious by examining the Manhattan plots. All four behavior traits display totally different SNPs associated with large effects on behavior. For general temperament there are two distinct peaks on Bos Taurus chromosome (**BTA**) 4 and BTA8 (Figure 1). For the obviously similar trait milking

temperament (Figure 2) there are no peaks on these chromosomes but an accumulation of SNPs on BTA14. A further region with large effect on aggressiveness is found on BTA8 but this region is located in the middle of the chromosome, whereas the region on BTA8 influencing general temperament is located at the end of BTA8. For rank order in herd (Figure 4), there are accumulations of SNPs on BTA1, BTA6, BTA18, and BTA19. None of the other behavior traits showed an accumulation of SNPs in these regions.

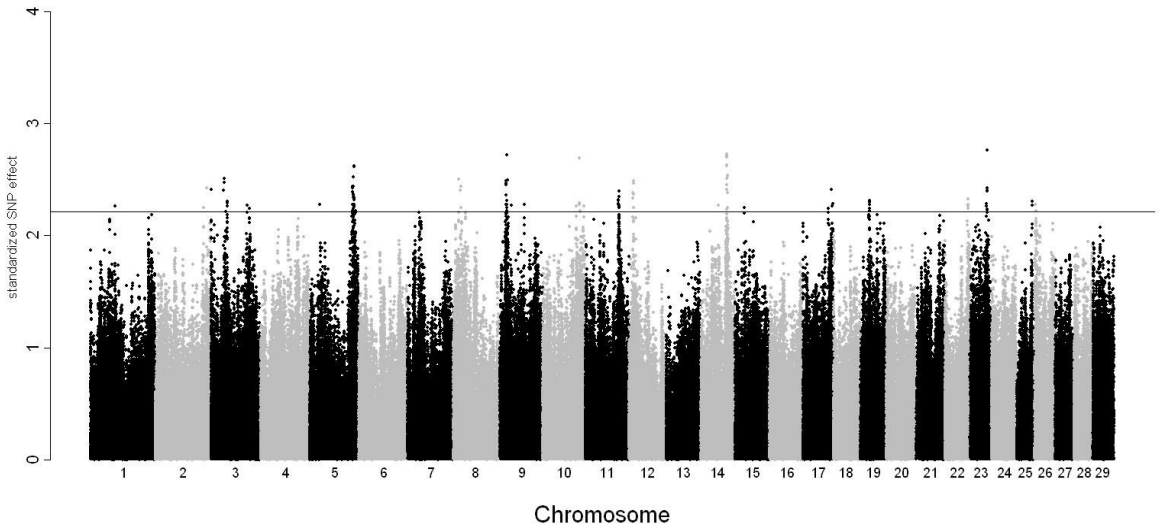

Figure 5: Manhattan plot for the trait milking speed. SNP above the horizontal line have a standardized effect of more than 4*SD.

Based on international EBV Guo et al. (2012) estimated SNP effects for milking speed in BS. The authors found a distinct peak in the Manhattan plot for milking speed on BTA6 which could not be confirmed in the present study (Figure 5). In contrast to Guo et al. (2012) peaks on BTA5, BTA9, BTA11, BTA12, and BTA14 were found in the Manhattan plot for milking speed. Some minor peaks for milking speed on BTA23, BTA25, and BTA26 were found in the present work, as well as in the study by Guo et al. (2012). As described in chapter 2, there is a wide range of methods used to measure milking speed: Exact measurement of milking speed in kg/min with a technical device, scoring on a subjective scale of $1-5$, or on a subjective scale of $1-9$, scoring by an independent person or scoring by the farmer. EBVs from different countries based on all these different methods contribute to the international EBVs used in the study of Guo et al. (2012) for estimation of SNP effects. In contrast, the SNP effects estimated in the present study are

based on EBVs derived from subjectively scoring by farmers (scale 1 – 6). These methodic differences in data assessment might explain some of the differences between this work and results of Guo et al. (2012).

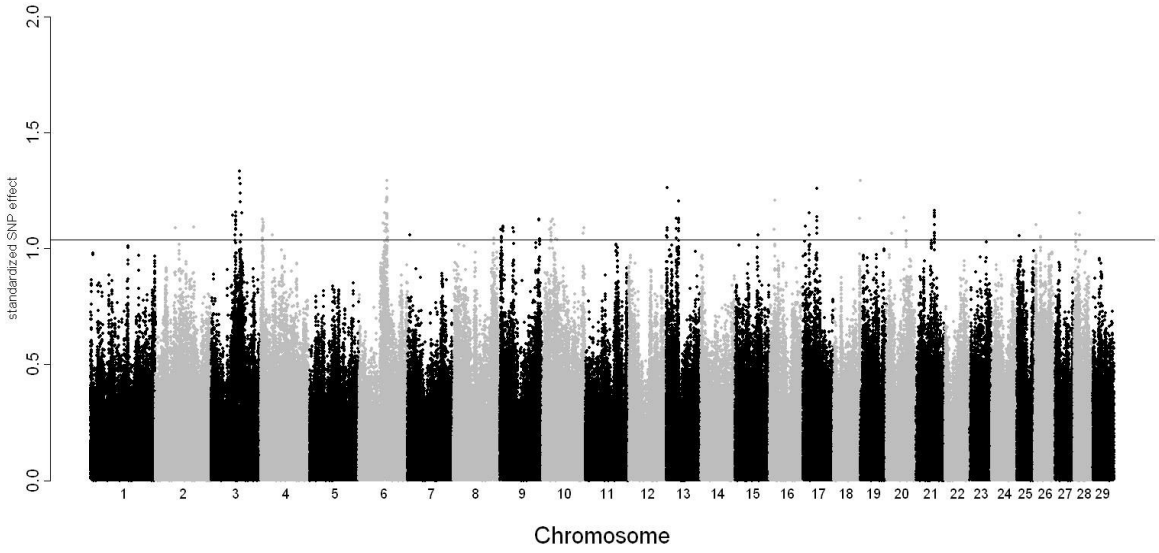

Figure 6: Manhattan plot for the trait udder depth. SNP above the horizontal line have a standardized effect of more than 4*SD.

With use of microsatellites Schrooten et al. (2000) and Lund et al. (2008) found a quantitative trait loci (**QTL**) with significant influence on fore udder attachment at the beginning of BTA13. These findings are supported by the current work. As shown in Figure 6, there is a region at the beginning of BTA13 with an aggregation of SNPs associated with udder depth. However, this peak is not as obvious as the peaks on BTA3 and BTA6 for udder depth. It should be considered that Schrooten et al. (2000) and Lund et al. (2008) used different methods to detect QTL (microsatellites), and that fore udder attachment is a different trait than udder depth. The authors also used Holstein Frisian and Danish Holstein cattle rather than BS.

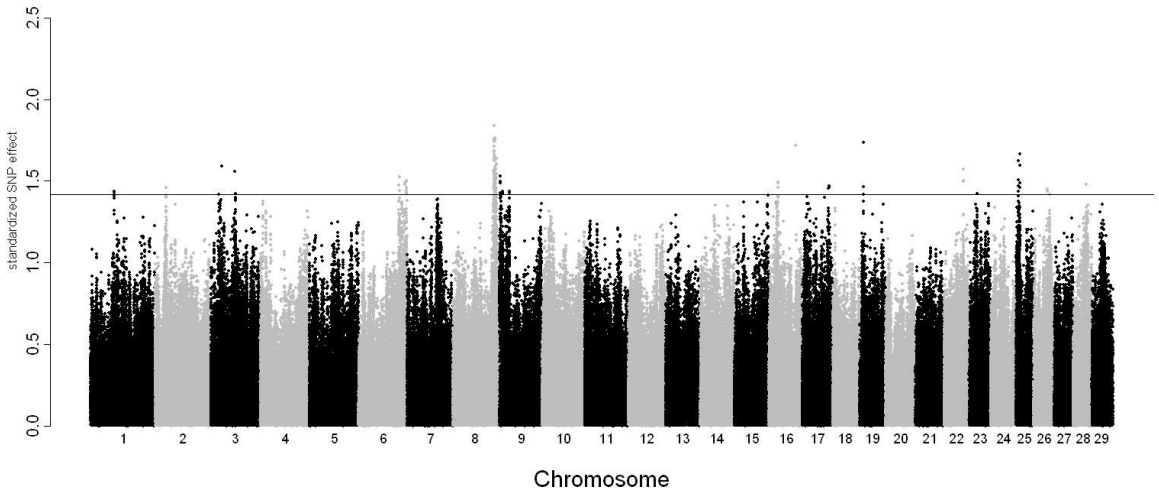

Figure 7: Manhattan plot for the trait position of labia. SNP above the horizontal line have a standardized effect of more than 4*SD.

As explained in chapter 2, the trait position of labia is a novel conformation trait which is associated with urovagina. As this trait was analyzed genetically for the first time in this study, there are no results from literature to compare with. Nevertheless, it is obvious that the position of labia is strongly influenced by connective tissue and fatty tissue around the labia. So it might be appropriate to compare the Manhattan plot of position of labia with Manhattan plots of other traits that are related to connective tissue like udder conformation traits. This comparison might also be justified by the fact that position of labia has a heritability of 0.28, which is in the range of heritabilities for udder conformation traits of 0.21 – 0.28 (chapter 2). In the present study, a distinct peak for position of labia was found on BTA25 (Figure 7). Schrooten et al. (2000) also found a region on BTA25 with influence on fore udder attachment and udder at all. In the current study also a more obvious peak was found on BTA8. However this peak cannot be explained from literature.

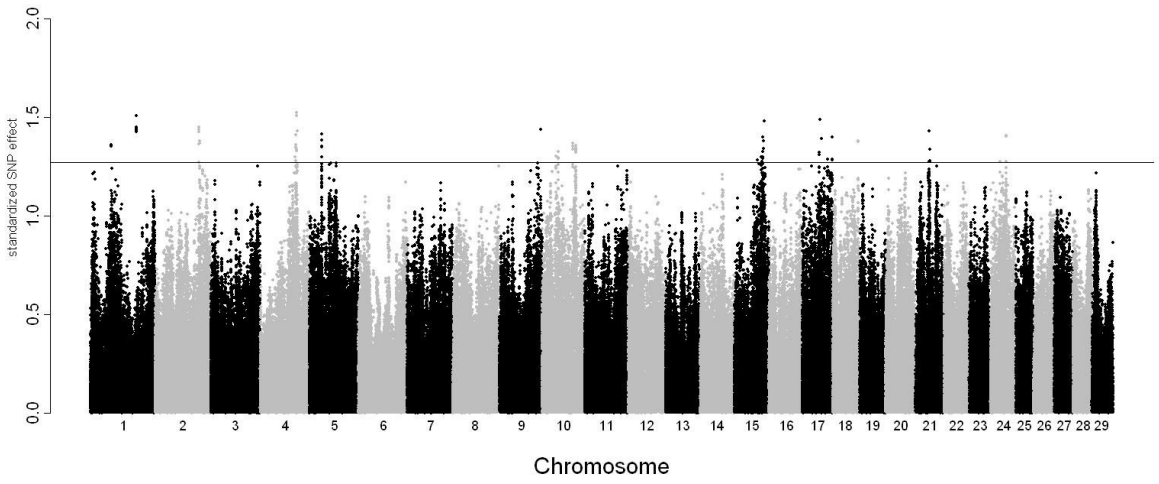

Figure 8: Manhattan plot for the trait days to first heat. SNP above the horizontal line have a standardized effect of more than 4*SD.

The trait number of days from calving to the first ovulation investigated by Hawken et al. (2012) is very similar to our trait days to first heat. While Hawken et al. (2012) found distinct peaks in the Manhattan plots on chromosome 3 and chromosome 14 for Brahman and on BTA5 and BTA16 for a tropical breed, both under tropical conditions. In the present work large peaks were found on BTA4, BTA15, and BTA21 for BS cattle under European conditions (Figure 8). For BS only a minor peak was found on BTA5. It has to be considered that Brahman belongs to a different subspecies (Bos Indicus) than BS (Bos Taurus). Fertility in a dairy breed might also be a different trait than fertility in a beef cattle breed. In dairy cattle there is a strong competition for nutrients between milk production and fertility shortly after calving. Due to lower milk performance in beef cattle more energy is available for fertility in these breeds, and thus other genes may play a role in beef cattle compared to dairy cattle. In warm and moist tropical climate, fertility might also be influenced by other genes than in moderate European climate. Hawken et al. (2012) further pointed out that some SNPs significantly associated with a trait in one breed, were not associated with the trait in the other breed. This could indicate that there is a breed difference for the SNPs associated with fertility traits, which might explain the differences between the results represented here and the results of Hawken et al. (2012).

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Acknowledgements

The authors gratefully acknowledge co-funding from the European Commission, under the Seventh Framework Program for Research and Technological Development, for the Collaborative Project LowInputBreeds (Grant agreement No 222623). However, the views expressed by the authors do not necessarily reflect the views of the European Commission, nor do they in any way anticipate the Commission's future policy in this area.

It is not only financial support that contributes to the success of a PhD thesis, it is also personal support that contributes to the success, and this is actually the more important form of support. So I would particularly like to thank all those people who accompanied me in the last three and a half years:

I would like to thank:

Prof. Dr. Simianer for offering me to work in his group and for acting as main supervisor

Prof. Dr. Sven König for taking over the co-reference.

Prof. Dr. Dr. Matthias Gauly for acting as third supervisor.

Malena Erbe and Reza Sharifi for Your assistance with ASReml and GNU R and for always being willing to discuss my results.

Beat Bapst and Birgit Gredler from QUALITAS AG, Zug and Anna Bieber from FiBL, Frick for answering all my questions concerning milk production and Brown Swiss breeding in Switzerland, for preparing genotypes and for collecting phenotypes.

Ganz herzlich bedanke ich mich…

… bei allen Freunden, deren Hochzeiten ich verpasst habe, weil ich mit der Anfertigung dieser Arbeite beschäftigt war: Christian und Katrin, Christel und Friedhelm, Dennis und Andrea. Zur Silberhochzeit bin ich dann wieder dabei ;-)

… bei allen meinen Freunden aus dem Institut für Tierzucht und Haustiergenetik: Tine, Lilly, Vivian, Lea, Florian, Dani, Björn, Mazahr, Hannah, Verena, Susanne und Friederike. Ohne Euch hätte die Promotionszeit nicht einmal halb so viel Spaß gemacht.

… bei allen meinen Freunden aus der Göttinger Zeit: Nadine, Chrissi, Simone, Harald, Meini, Markus, Anna und Andreas. Ihr habt dazu beigetragen aus der Zeit in Göttingen die (bisher) schönste meines Lebens zu machen.

… bei meinen Eltern, dafür dass Ihr mich zu dem gemacht habt was ich bin.