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**MARKER-ASSISTED ESTIMATION OF PEDIGREE ERRORS AND
QTL MAPPING IN THE GERMAN ANGELN DAIRY CATTLE
POPULATION**

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Meiner Familie

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INTRODUCTION

With the assistance of genetic markers, several genes accounting for genetically complex traits (quantitative trait loci, QTL) have been mapped in different dairy cattle populations in the last decade. QTL for the milk production traits milk yield, fat yield and content as well as protein yield and content have been mapped in several dairy cattle populations (reviewed by Bovenhuis and Schrooten, 2002). These mapping studies were based on two typical half-sib designs, either the granddaughter design or the daughter design (Weller et al., 1990). Especially in small dairy cattle populations, e.g., the Angeln dairy cattle population, the daughter design is a favourable experimental design because of the lack of large male paternal half-sib groups.

The **objectives of this thesis** were the mapping of QTL for milk production traits in the German Angeln dairy cattle population with special emphasis on the mapping process of a QTL on BTA14 and a QTL on BTA6, respectively. Additionally, the genotypic results were used for a marker-assisted estimation of wrong sire information in the Angeln breed. Five paternal female half-sib families with a total of 805 daughters were chosen for the genotyping process, because of their relatively large female half-sib groups.

In **chapter one** a marker-assisted estimation of wrong sire information was carried out using the genetic information of sixteen microsatellite markers. These markers were distributed over five different bovine chromosomes (BTA6, 14, 16, 18, and 27) and were chosen because of their highly polymorphic character. Allele frequencies and exclusion probabilities of each included marker were estimated. Additionally, the impact of wrong and missing sire information on the reliability of the estimated breeding values and on the genetic gain was investigated with deterministic simulations.

In the last decade, several studies have reported the evidence and the effects of a QTL on BTA14 affecting milk production traits. A nonconservative mutation (*K232A*) of lysine to alanine in the *DGATI* gene was characterised in two independent studies (Grisart et al., 2002; Winter et al., 2002). The diallelic *DGATI* gene is segregating in the centromeric region on BTA14 and was identified as a candidate gene for milk production traits, especially for fat yield and content. Recently, Kühn et al. (2004) investigated an additional source of variation which is associated with an increasing amount of fat content in the *DGATI* VNTR (variable number of tandem repeats) promoter region. Beyond the *DGATI* gene different studies

investigated the effects of the alleles at the casein cluster and especially at the *CSN1S1* promoter on BTA6. The casein cluster showed effects especially on the both milk protein traits (protein yield and content). In **chapter two** the frequencies and the effects of the alleles at the *DGATI K232A* mutation and at the *DGATI* promoter VNTR were analysed. Furthermore, the frequencies and the effects of the alleles at the *CSN1S1* promoter on BTA6 (Prinzenberg et al., 2003) were investigated.

The mapping of QTL affecting milk production traits was a main objective of several marker-assisted investigations in different dairy cattle populations. Some of these traits, e.g., milk yield, are of a complex nature and the QTL are therefore difficult to explain genetically. The mapping of trait related QTL may be a possibility to understand and to explain the physiological background of these traits in a better way. In **chapter three** a QTL analysis on five chromosomes and 43 markers, mostly microsatellites, for the milk production traits milk, fat, protein, lactose, and milk energy yield, and fat, protein, lactose, and milk energy content, and somatic cell score was conducted.

This thesis ends with a general discussion in **chapter four**.

REFERENCES

- Bovenhuis, H., and C. Schrooten. 2002. Quantitative trait loci for milk production traits in dairy cattle. Proc. 7th World Congr. Genet. Appl. Livestock Prod. No. 09–07, Montpellier, France.
- Grisart, B., W. Coppieters, F. Farnir, L. Karim, C. Ford, P. Berzi, N. Cambisano, M. Mni, S. Reid, P. Simon, R. Spelman, M. Georges, and R. Snell. 2002. Positional candidate cloning of a QTL in dairy cattle: Identification of a missense mutation in the bovine *DGATI* gene with major effect on milk yield and composition. *Genome Res.* 12:222–231.
- Kühn, Ch., G. Thaller, A. Winter, O. R. P. Bininda-Emonds, B. Kaupe, G. Erhardt, J. Bennewitz, M. Schwerin, and R. Fries. 2004. Evidence for multiple alleles at the *DGATI* locus better explains a quantitative trait locus with major effect on milk fat content in cattle. *Genetics* 167:1873–1881.
- Prinzenberg, E.-M., C. Weimann, H. Brandt, J. Bennewitz, E. Kalm, M. Schwerin, and G. Erhardt. 2003. Polymorphism of the bovine *CSN1S1* promoter: linkage mapping, intragenic haplotypes, and effects on milk production traits. *J. Dairy Sci.* 86:2696–2705.

- Weller, J. I., Y. Kashi, and M. Soller. 1990. Power of daughter and granddaughter designs for determining linkage between marker loci and quantitative trait loci in dairy cattle. *J. Dairy Sci.* 73:2525–2537.
- Winter, A., W. Krämer, F. A. O. Werner, S. Kollers, S. Kata, G. Durstewitz, J. Buitkamp, J. E. Womack, G. Thaller, and R. Fries. 2002. Association of a lysine-232/alanine polymorphism in a bovine gene encoding acyl-CoA:diacylglycerol acyltransferase (*DGATI*) with variation at a quantitative trait locus for milk fat content. *Proc. Natl. Acad. Sci. USA* 99(14):9300–9305.

CHAPTER ONE

Wrong and missing sire information affects genetic gain in the Angeln dairy cattle population

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ABSTRACT

In the present study, molecular genetic markers were used to help estimate the degree of wrong sire information in the German Angeln dairy cattle population. Sixteen polymorphic microsatellite markers were genotyped on 5 different paternal half-sib families with a total of 805 daughters. For the genotyping process, blood samples of the daughters and semen samples of the sires were used. Allelic frequencies and exclusion probabilities were estimated. The simultaneous effect of wrong (WSI) and missing sire information (MSI) on the reliability of estimated breeding values and on the genetic gain was investigated using deterministic simulations. For these simulations, different values for the number of daughters per sire, heritability, WSI, and MSI were chosen. The estimated proportion of the WSI was 7% in the German Angeln dairy cattle population. The combined impact of WSI and MSI on the genetic gain was relatively large, especially in the case of a small progeny size per sire and lower values of heritability. The impact of WSI was more harmful than MSI on the response to selection.

(Key words: exclusion probability, genetic response, missing sire information, wrong sire information)

Abbreviation key: CPE = combined exclusion probability, EBV = estimated breeding value, MSI = missing sire information, PE = exclusion probability, WSI = wrong sire information.

1. INTRODUCTION

Wrong sire information (WSI) is a well-known problem in the estimation of breeding values for dairy cattle. Several studies estimated the proportion of WSI at 3 to 23% in the Holstein Friesian breed (Visscher et al., 2002; Ron et al., 2003; Weller et al., 2004). The presence of WSI reduces realized genetic response (relative to expected response) because the estimates of heritability are biased downward (Israel and Weller, 2000; Banos et al., 2001; Visscher et al., 2002). Additionally, WSI might have a more important effect on genetic gain of lowly heritable traits, because in this case, the impact of the pedigree information on the EBV using BLUP is higher. Christensen et al. (1982) and Weller et al. (2004) mentioned different reasons for paternity errors, which can originate from AI companies, recording by the farmer, or genotyping service, and can arise because of human or technical error.

The second source of pedigree errors is missing sire information (MSI). In contrast to WSI, until now there has been comparatively little information on the extent and impact of this source of error, but as discussed by Harder et al. (2005), the proportion of MSI can be substantial. Harder et al. (2005) pointed out that MSI influences the variance of estimated sire breeding values and reduces the response to selection.

The objective of the present study was to estimate the proportion of wrong sire information in the Angeln dairy cattle population using molecular marker information. Furthermore, the consequences of WSI and MSI on the genetic gain were investigated by deterministic simulation.

2. MATERIAL AND METHODS

2.1 Field data

The red Angeln breed is a small breed located in the northern part of Germany. Since 1960, different red cattle breeds have been crossed with the Angeln breed. Savaş et al. (1998) reported that 40% of the Angeln population had proportions ranging from 13 to 37% of genetics from other cattle breeds such as Red-Holstein, Swedish Red and White, and Finnish Ayrshire. The breed is listed in the database of the European Association of Animal Production (EAAP, 2004), where additional information about it can be found. In the present study, a daughter design was used (Weller et al., 1990) in which 5 paternal half-sib families with a total of 805 daughters were selected. The family size ranged from 123 daughters in family 1 to 199 daughters in family 4; the average was 161 daughters per sire.

2.2 Genotyping process

In 2000, blood samples of the daughters were collected on 41 farms, and semen samples were taken from the 5 sires. No blood samples of the daughters' dams were available. The DNA was extracted using the silica-gel method (following Myakishev et al., 1995). The DNA extraction was carried out as follows: 325 µl of the whole blood (containing 50 mM EDTA) or semen was mixed with 650 µl bind mix in a 1.5-ml minicentrifuge tube. The combination was incubated in a hybridization oven at 37°C for 15 min (lysis), and the pellet was collected by centrifugation at 5000 rpm for 10 s in an Eppendorf centrifuge (Eppendorf AG, Hamburg, Germany). The supernatant was poured off. This procedure was conducted 3 times. The pellet was resuspended in 1.0 ml of guanidine solution by vortexing and was incubated in a hybridization oven (37°C). The supernatant was then poured off. The guanidine washing procedure and the incubation were then repeated. The pellet was resuspended in 1 ml of propanol wash, incubated in a hybridization oven and centrifuged at 5000 rpm for 10 s. The supernatant was then poured off. This washing procedure was conducted twice. Finally, the pellet was dissolved in 1 ml ethanol (once) and centrifuged; the supernatant was poured off. The probe was dried for 45 min under vacuum in an exsiccator. The pellet was then resuspended in 400 µl Tris-HCl-buffer (pH 8.0) and dissolved overnight at 4°C.

The 805 daughters and the 5 sires were genotyped for 16 microsatellite markers. These markers are located on 5 different chromosomes (BTA6, 14, 16, 18, and 27) and were selected from previously published bovine marker maps (USDA cattle genome marker maps: <http://www.marc.usda.gov/genome/genome.html>; INRA BOVMAP database: <http://locus.jouy.inra.fr/>). (The properties of the involved markers will be described in the Results section.). They were chosen because of their highly polymorphic character, and their genotypic information will be used in a subsequent QTL mapping project. The PCR for the microsatellite markers was completed on a MJ Research PTC-200 thermocycler (Global Medical Instrumentation Inc., Ramsey, MN). The electrophoresis of the fluorescent-labeled microsatellite markers was carried out using the MegaBACE 500 Analysis System (Amersham Biosciences Europe GmbH, Freiburg, Germany) and analyzed with the MegaBACE Genetic Profiler Software Suite v2.2 (Amersham Biosciences Europe GmbH). The genotypes were transferred into the ADRDB database (Reinsch, 1999) and checked for agreement with the Mendelian laws of inheritance with the program GENCHECK (Bennewitz et al., 2002). To exclude genotyping mistakes, animals involved in conflicts were genotyped for a second time. However, most conflicts (i.e., a violation of the Mendelian laws of inheritance) remained after the second genotyping. Within the set of conflicts that remained

unsolved, a paternity was declared wrong if a conflict between a daughter and its putative sire was observed at ≥ 3 loci.

2.3 Marker characteristics and estimation of pedigree errors

The allelic frequencies were estimated by maximum likelihood with the following log-likelihood function (Brka et al., 2002):

$$\ln L = \sum_{i=1}^n n_i \ln p_i + \sum_{i=1}^n \sum_{j=i+1}^n \frac{1}{2} n_{ij} \ln(p_i + p_j),$$

with $p_n = 1 - \sum_{i=1}^{n-1} p_i$, where n = number of the different alleles at the particular marker,

n_i = number of allele i from the founder animals (i.e., either from founder sires or unequivocally descending from unknown dams), and n_{ij} = number of half-sibs that share the same heterozygous genotype with their sire. This formula is tailored to a half-sib structure because it allows the use of genotypic information of those daughters with alleles whose paternal origin cannot be unequivocally determined.

For a single locus, the exclusion probability is defined as the probability that a putative conflict between a sire and a daughter occurs in the case of a true nonpaternity and is calculated from the frequencies of the different marker alleles. Exclusion probabilities for each marker were calculated for the whole population (PE_{pop}) and each single family ($PE_{fam(i)}$). Following the S-notation given by Dodds et al. (1996), the PE_{pop} was estimated for a single locus as

$$PE_{pop} = 1 - 4S_2 + 4S_3 - 3S_4 + 2S_2^2$$

with $S_t = \sum_{i=1}^n p_i^t$, where p_i = frequency of allele i , n = number of distinct alleles, and

t = arbitrary non-negative integer (Dodds et al., 1996).

The family-specific exclusion probability ($PE_{fam(i)}$) was estimated following Ron et al. (1996):

$$PE_{fam(i)} = (1 - q_i)^2$$

where q_i is the sum of the frequencies of the 2 alleles of sire i .

Extending this to multiple loci, the method described by Ron et al. (1996) was followed to calculate a combined exclusion probability for the whole population (CPE_{pop}):

$$CPE_{pop} = 1 - \prod_{j=1}^m (1 - PE_{pop(j)})$$

where m = number of genotyped loci and $PE_{pop(j)}$ = exclusion probability for the population at locus j . The combined exclusion probability for each family ($CPE_{fam(i)}$) was calculated as follows:

$$CPE_{fam(i)} = 1 - \prod_{j=1}^m (1 - PE_{fam(i)j})$$

where $PE_{fam(i)j}$ = exclusion probability for family i at the locus j .

The power of this study was defined as the probability of detecting a nonpaternity given a random case of nonpaternity. In a first step, the probability of nonpaternity in the case of zero to 3 loci showing a conflict was calculated. In the next step, the power was calculated as the sum of the probabilities showing a conflict at more >2 loci given a random case of nonpaternity. These probabilities can be calculated from the individual marker exclusion probabilities. One minus these probabilities of all included loci is equal to the power of this study.

2.4 Effect of pedigree errors on selection response

Following Mrode (1996), the selection response (ΔG) per generation can be defined as

$$\Delta G = i \times \sqrt{R} \times \sigma_a,$$

where i = intensity of selection, σ_a = additive genetic standard deviation, and R = reliability of the estimated breeding value; therefore, \sqrt{R} represents its accuracy.

Assuming that only progeny records contribute to the EBV of a sire, R is defined as (Mrode, 1996)

$$R = \frac{N}{(N + \lambda)}, \text{ where } \lambda = \frac{(1 - t)}{t} \quad (1)$$

where N = number of potential progenies per sire and t = intraclass correlation, which is one-quarter of the heritability in the case of a half-sib progeny group.

The impact of WSI and MSI can be illustrated by a small example. Assume a population consisting of 2 paternal half-sib groups, each of 100 daughters. Further, assume a WSI and an MSI, both of 10%. In this case, 90 daughters are assigned to each sire (MSI = 0.10), but only 81 daughters are correctly assigned to each sire (WSI = 0.10) and 9 daughters are assigned to the wrong sire, respectively. Similar to Visscher et al. (2002) it was assumed that i and σ_a were not affected by WSI. Therefore, the impact of WSI and MSI on the selection response can be expressed as

$$\Delta G \propto \sqrt{R_e}, \text{ where } R_e = \frac{(1 - \text{MSI}) \times N}{((1 - \text{MSI}) \times N) + \lambda_e} \text{ and } \lambda_e = \frac{1 - (1 - \text{WSI}) \times t}{(1 - \text{WSI})^2 \times t}. \quad (2)$$

Subscript e denotes for pedigree errors. The efficiency of a breeding plan with respect to the genetic gain with pedigree errors relative to a situation with no pedigree errors can be estimated as (Visscher et al., 2002)

$$E_G = \sqrt{\frac{R_e}{R}}, \quad (3)$$

where R is the reliability without pedigree errors.

To investigate the impact of different WSI and MSI on the response to selection, the efficiency was calculated for a number of configurations that might reflect a general progeny dairy cattle breeding scheme based on progeny testing, including the situation that can be found in the Angeln population. Different values for heritability ($h^2 = 0.10, 0.25, \text{ and } 0.50$) and for the progeny group size ($N = 10, 50, \text{ and } 100$) were used in the calculations. The proportion of WSI was varied in 6 steps ($\text{WSI} = 0.05, 0.07, 0.10, 0.15, 0.20, \text{ and } 0.30$), and for MSI, 4 different values were chosen ($\text{MSI} = 0.10, 0.20, 0.30, \text{ and } 0.40$).

Additionally, as suggested by an anonymous reviewer, the different impacts of MSI and WSI on the efficiency can be derived analytically by taking the first derivative of the square of Equation (1) with respect to $(1 - \text{MSI})$ and to $(1 - \text{WSI})$, respectively (see Appendix).

3. RESULTS AND DISCUSSION

3.1 Heterozygosity and exclusion probability

Table 1 has the observed heterozygosities and the exclusion probability for the different markers. The number of alleles varied from 7 for marker BP7 to 13 for marker TGLA227. The heterozygosity of the 16 different markers ranged between 0.49 for marker INRA134 and 0.80 for markers TGLA227 and BMS2639. The exclusion probability increased with increasing heterozygosity, although this relationship was not strictly monotonic. Accordingly, the lowest exclusion probability was obtained for marker INRA134 ($\text{PE}_{\text{pop}} = 0.13$), and the highest probabilities were observed for markers TGLA227 and BMS2639 ($\text{PE}_{\text{pop}} = 0.44$). The combined exclusion probability for all 16 markers was 0.999. These results are in accordance with different studies, which pointed out that a high heterozygosity is better for paternity verification, because markers with low heterozygosity are the reason for underestimating misidentification rates (Dodds et al., 1996; Ron et al., 1996; Visscher et al., 2002).

Table 1: Degree of observed heterozygosity, exclusion probability (PE_{pop}), and combined exclusion probability (CPE_{pop}) in the Angeln population

Marker	Alleles (No.)	Heterozygosity	PE_{pop}
BP7	7	0.67	0.26
RM209	12	0.63	0.24
BMS1675	8	0.77	0.39
BM3507	11	0.78	0.43
CSSM028	11	0.74	0.34
BM4513	9	0.69	0.29
TGLA227	13	0.80	0.44
HUJ625	8	0.76	0.37
INRA048	9	0.76	0.36
RM180	9	0.65	0.25
DIK082	11	0.79	0.42
BM6425	10	0.72	0.32
BMC4203	10	0.73	0.33
BM6507	10	0.65	0.25
BMS2639	12	0.80	0.44
INRA134	8	0.49	0.13
CPE_{pop}			0.999

In addition, the exclusion probabilities within single families ($PE_{fam(i)}$) were of interest (Table 2). These exclusion probabilities ranged between 0.0004 and 0.94 for the different markers and the different families. Table 2 shows that markers with a high PE_{pop} (e.g., BMS2639) did not automatically have a high exclusion probability within each of the 5 families. Furthermore, it was shown that the $PE_{fam(i)}$ values for a given marker varied substantially within the other families (e.g., BM3507).

Table 2: Exclusion probability ($PE_{fam(i)}$) and combined exclusion probability ($CPE_{fam(i)}$) within different families in the Angeln population

	N_{gen}^1	$PE_{fam(i)}$				
		Family 1	Family 2	Family 3	Family 4	Family 5
BP7	732	0.0004	0.12	0.0004	0.08	0.0004
RM209	685	0.77	0.13	0.09	0.08	0.03
BMS1675	604	0.64	0.55	0.19	0.24	0.24
BM3507	729	0.25	0.34	0.64	0.49	0.92
CSSM028	721	0.09	0.10	0.10	0.40	0.26
BM4513	739	0.07	0.07	0.22	0.07	0.07
TGLA227	725	0.31	0.40	0.31	0.23	0.94
HUJ625	735	0.46	0.61	0.12	0.74	0.28
INRA048	694	0.52	0.52	0.29	0.58	0.30
RM180	734	0.004	0.07	0.07	0.14	0.004
DIK082	736	0.34	0.14	0.23	0.61	0.34
BM6425	735	0.41	0.07	0.10	0.29	0.29
BMC4203	740	0.09	0.61	0.31	0.41	0.09
BM6507	738	0.11	0.07	0.52	0.11	0.004
BMS2639	743	0.18	0.14	0.21	0.46	0.35
INRA134	650	0.16	0.16	0.05	0.16	0.05
CPE_{fam}		0.998	0.996	0.987	0.999	0.999

¹ N_{gen} is the total number of genotyped daughters for the respective marker.

Adopting the classical test statistic theory to the problem of a marker-based detection of WSI, a Type I error occurs if a correct sire is erroneously declared as wrong because of genotyping mistakes, for example. A Type II error occurs if a wrong sire is not declared as one. Further, as previously mentioned, the power of the study is the probability to detect a wrong sire as being wrong. For the calculation of the power of this study, the results of Table 1 were used. The power would be the highest (> 0.99) if a wrong paternity was declared when at least one locus showed a conflict. However, this would result in a relatively high Type I error rate, although measurement of the Type I error is not possible. In the case of 2 loci showing a conflict, the power would be 0.94. To find a compromise between the power and the Type I

error rate, a wrong paternity was declared if a conflict was observed at ≥ 3 loci, corresponding to a power of 0.83.

3.2 Proportion of wrong sire information

Table 3 shows the putative WSI of the Angeln population for varying number of conflicts. Of 805 animals 10.8% showed conflicts at ≥ 1 loci. None of the animals showed conflicts for ≥ 8 of the 16 markers. The final WSI in the Angeln population was estimated to be 7%, bearing in mind that an unknown proportion of the WSI might be erroneously declared as WSI (i.e., reflect a Type I error). For the 5 different families, the estimated proportions of WSI were 4.07, 5.56, 11.11, 11.56, and 5.67%, respectively. The estimated WSI in the Angeln population is in accordance with the literature reports for other breeds (Visscher et al., 2002; Ron et al., 2003; Weller et al., 2004).

Table 3: Putative wrong sire information (WSI) for a varied number of conflicts in the Angeln population (Note that the final choice of the number of conflicts for declaring a WSI was ≥ 3 loci, which corresponds to a final WSI of 7% in the Angeln population)

Markers showing conflicts	Daughters (No.)	Putative WSI	SE ¹
≥ 1	87	0.108	0.0109
≥ 2	66	0.082	0.0097
≥ 3	56	0.070	0.0090
≥ 4	46	0.057	0.0082
≥ 5	36	0.045	0.0073
≥ 6	20	0.025	0.0055
≥ 7	5	0.006	0.0027
≥ 8	1	0.001	0.0011

¹SE = $\sqrt{\text{WSI}(1 - \text{WSI})/N}$, with N = 805 daughters.

3.3 Impact of WSI and MSI on genetic gain

The influence of different values for WSI and MSI from 0 to 30% on reliability for the case of 100 daughters per sire and the values for the heritability of $h^2 = 0.10$ and of $h^2 = 0.25$ are presented in Figure 1. With an increase in WSI and MSI, the reliability decreased. The effect of WSI and MSI was more detrimental in the case of lower heritability.

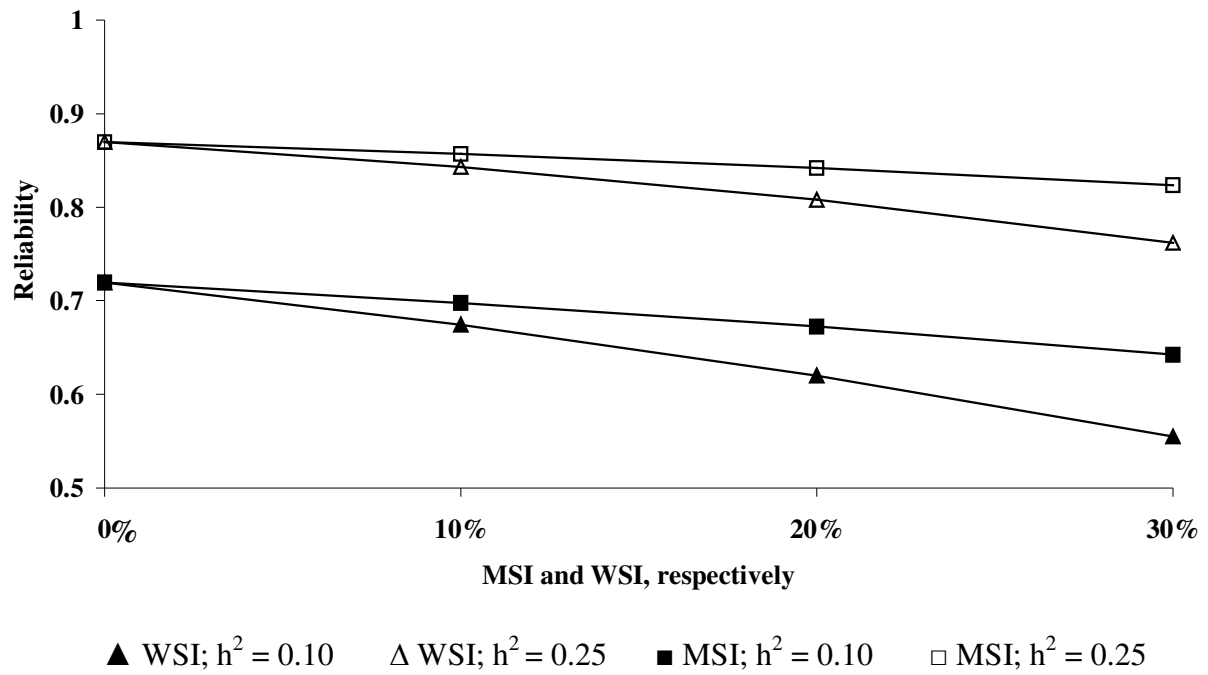


Figure 1: Reliability for the case of 100 daughters per sire, a heritability of $h^2 = 0.10$ and $h^2 = 0.25$, and different values (0 to 30%) for wrong sire information (WSI) and missing sire information (MSI).

The impact of the different values of the heritability and the number of daughters per sire are presented in Table 4. These results represent the putative situation in the Angeln population, i.e., a WSI of 7% and a MSI of 10% (F. Reinhardt, VIT Verden, Germany, personal communication).

Table 4: Reliability and efficiency of sire evaluation when there is 10% missing sire information (MSI¹) and 7% wrong sire information (WSI¹) for 3 levels of heritability and progeny size

Heritability	Daughter group size	Reliability	Efficiency
0.10	10	0.17	0.90
	50	0.50	0.94
	100	0.67	0.96
0.25	10	0.34	0.92
	50	0.72	0.97
	100	0.84	0.98
0.50	10	0.52	0.94
	50	0.85	0.98
	100	0.92	0.99

¹ These values might reflect the situation that can be found in the Angeln population.

Other studies in the literature pointed out that WSI influenced the genetic gain downward because of the downward bias of the heritability and the lower number of progeny with correct pedigree information (Israel and Weller, 2000; Banos et al., 2001; Visscher et al., 2002). In contrast to WSI, in the study of Harder et al. (2005), MSI did not affect the estimated additive genetic variance, but the decrease in progeny size reduced the reliability of the EBV. In addition, MSI had an effect on the mean square error of the fixed effects estimate, because the estimation of the variance-covariance-matrix of the observations of the cows was incorrect for cows with MSI. Nevertheless, Harder et al. (2005) showed that it was important to have the daughters with MSI included in the estimation; otherwise the mean square error of the fixed effects would be even greater.

The present study pointed out that WSI and MSI combined their effects on the genetic gain. The calculations showed that the impact of WSI was more harmful than that of MSI. The first derivation of Equation (1) showed, either with respect to $(1 - \text{MSI})$ or with respect to $(1 - \text{WSI})$, that the effect on the efficiency of WSI was around 1.4 times more harmful as MSI, assuming large progeny groups (see Appendix). Increasing the number of daughters per sire decreases the influence of WSI and MSI, especially in the case of a low heritability (Table 4). The results of the calculations presented in Figure 1 and Table 4 are in agreement with Van Vleck (1970a, 1970b) and Christensen et al. (1982), who concluded that a trait with a lower heritability and $\text{WSI} > 0$ had higher losses in the genetic gain than traits with a higher value of

heritability. Harder et al. (2005) showed similar results for MSI, which reduced the genetic gain, especially for traits with low heritabilities.

4. CONCLUSIONS

Microsatellite markers are suitable tools for the determination of WSI. In the German Angeln dairy cattle population, the estimated proportion of WSI was 7%. The estimation of WSI was done with the assistance of 16 microsatellite markers and accepting 3 conflicts as an indication of WSI. The power of this study, in the case of ≥ 3 loci showing a conflict, was 0.83. Additionally, it was shown that WSI and MSI had an influence on reliability and on genetic gain in the Angeln dairy cattle breed, and combined their effect on the genetic gain. The impact of WSI on the efficiency is around 1.4 times more harmful than the impact of MSI. If a reduction in the loss of genetic gain is desired, especially for lowly heritable traits caused by incorrect paternity, breeding organizations must check their recording and verification systems to decrease the proportion of wrong and missing pedigree records or to increase the number of daughters per sire.

APPENDIX

As shown in Equation 3 of the main text, efficiency (E_G) is defined as $E_G = \sqrt{\frac{R_e}{R}}$, where R_e = reliability with pedigree errors (Equation 2 in the main text) and R = reliability without pedigree errors (Equation 1 in the main text).

Using Equations 1, 2, and 3, and defining $x = (1 - \text{MSI})$ and $y = (1 - \text{WSI})$, the square of the efficiency is given by

$$E_G^2 = \frac{xy^2[(N-1)t+1]}{yt(xyN-1)+1} \quad (\text{A1})$$

with N = number of potential progenies per sire and t = intraclass correlation.

Taking the first derivative of Equation A1 with respect to x yields with some algebraic operations:

$$\frac{\delta E_G^2}{\delta x} = \frac{E_G^2}{x} - \frac{y^2 N t \times E_G^2}{yt(xyN-1)+1} \quad (\text{A2})$$

Setting $x = y = E_G = 1$ (i.e., no pedigree errors) results in

$$\frac{\delta E_G^2}{\delta x} = 1 - \frac{Nt}{t(N-1)+1} = 1 - R. \quad (\text{A3})$$

Taking the first derivative of Equation A1 with respect to y yields, after some algebraic operations,

$$\frac{\delta E_G^2}{\delta y} = \frac{2E_G^2}{y} - \frac{E_G^2 \times [t(2xyN - 1)]}{yt(xyN - 1) + 1}. \quad (\text{A4})$$

Setting $x = y = E_G = 1$ (i.e., no pedigree errors) results in

$$\frac{\delta E_G^2}{\delta y} = 2 - \frac{t(2N - 1)}{t(N - 1) + 1} = 2(1 - R) - \frac{t}{t(N - 1) + 1}. \quad (\text{A5})$$

Equations A3 and A5 show that the increase of MSI and WSI (i.e., a decrease of x and y) results in a loss of E_G^2 , that is equal for $(1 - R)$ and $(2(1 - R) - \frac{t}{t(N - 1) + 1})$, respectively.

Hence, for large N , the impact of WSI on E_G is around 1.4 (i.e., $\sqrt{2}$) times as harmful as MSI.

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REFERENCES

- Banos, G., G. R. Wiggins, and R. L. Powell. 2001. Impact of paternity errors in cow identification on genetic evaluations and international comparisons. *J. Dairy Sci.* 84(11):2523–2529.
- Bennewitz, J., N. Reinsch, and E. Kalm. 2002. GENCHECK: A program for consistency checking and derivation of genotypes at co-dominant and dominant loci. *J. Anim. Breed. Genet.* 119(5):350–360.
- Brka, M., N. Reinsch, and E. Kalm. 2002. Is there linkage between supernumerary teats in cattle and BTA3 markers? *Arch. Tierz.* 45(5):429–432.
- Christensen, L. G., P. Madsen, and J. Petersen. 1982. The influence of incorrect sire-identification on the estimates of genetic parameters and breeding values. *Proc. 2nd World Congr. Genet. Appl. Livest. Prod., Madrid, Spain VII*:200–208.
- Dodds, K. G., M. L. Tate, J. C. McEwan, and A. M. Crawford. 1996. Exclusion probabilities for pedigree testing farm animals. *Theor. Appl. Genet.* 92:966–975.
- EAAP. 2004. Online. <http://www.tiho-hannover.de/einricht/zucht/eaap/descript/8.htm>.
- Harder, B., J. Bennewitz, N. Reinsch, M. Mayer, and E. Kalm. 2005. Effect of missing sire information on genetic evaluation. *Arch. Tierz.* 48(1-3):219–232.

- Israel, C., and J. I. Weller. 2000. Effect of misidentification on genetic gain and estimation of breeding value in dairy cattle populations. *J. Dairy Sci.* 83(1):181–187.
- Mrode, R. A. 1996. *Linear Models for the Prediction of Animal Breeding Values*. CAB INTERNATIONAL Wallingford Oxon OX10 8DE, UK.
- Myakishev, M. V., G. I. Kapanadze, G. O. Shaikhayev, G. P. Georgiev, and D. R. Beritashvili. 1995. Extraction of DNA from the whole blood by silica gel. Publication of Institute of Gene Biology, Moscow.
- Reinsch, N. 1999. A multiple-species, multiple-project database for genotypes at codominant loci. *J. Anim. Breed. Genet.* 116(5):425–435.
- Ron, M., Y. Blanc, M. Band, E. Ezra, and J. I. Weller. 1996. Misidentification rate in the Israeli dairy cattle population and its implications for genetic improvement. *J. Dairy Sci.* 79(4):676–681.
- Ron, M., R. Domochofsky, M. Golik, E. Seroussi, E. Ezra, C. Shturman, and J. I. Weller. 2003. Analysis of vaginal swabs for paternity testing and marker-assisted selection in cattle. *J. Dairy Sci.* 86(5):1818–1820.
- Savaş, T., N. Reinsch, and E. Kalm. 1998. Auswirkungen der Rassenzusammensetzung auf Merkmale der Tagesmilchmenge und auf die Zellzahl beim Angler Rind. *Arch. Tierz.* 41(3):201–209.
- Van Vleck, L. D. 1970a. Misidentification in estimating the paternal sib correlation. *J. Dairy Sci.* 53(10):1469–1474.
- Van Vleck, L. D. 1970b. Misidentification and sire evaluation. *J. Dairy Sci.* 53(12):1697–1702.
- Visscher, P. M., J. A. Woolliams, D. Smith, and J. L. Williams. 2002. Estimation of pedigree errors in the UK dairy population using microsatellite markers and the impact on selection. *J. Dairy Sci.* 85(9):2368–2375.
- Weller, J. I., Y. Kashi, and M. Soller. 1990. Power of daughter and granddaughter designs for determining linkage between marker loci and quantitative trait loci in dairy cattle. *J. Dairy Sci.* 73(9):2525–2537.
- Weller, J. I., E. Feldmesser, M. Golik, I. Tager-Cohen, R. Domochofsky, O. Alus, E. Ezra, and M. Ron. 2004. Factors affecting incorrect paternity assignment in the Israeli Holstein population. *J. Dairy Sci.* 87(8):2627–2640.

CHAPTER TWO

Characterisation of the *DGATI* mutations and the *CSN1S1* promoter in the German Angeln dairy cattle population

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ABSTRACT

The identification of QTL and genes with influence on milk production traits has been the objective of various mapping studies in the last decade. In the centromeric region of the bovine chromosome 14 the acyl-CoA:diacylglycerol acyltransferase1 gene (*DGATI*) has been identified as the most likely causative gene underlying a QTL for milk fat yield and content. Recently, a second polymorphism in the promoter of *DGATI* emerged as an additional source of variation. In this study, the frequencies and the effects of alleles at the *DGATI* *K232A* and at the *DGATI* promoter VNTR locus on BTA14, and of alleles at the *CSN1S1* (α_{s1} -casein encoding gene) promoter on BTA6 in the German Angeln dairy cattle population were investigated. Analysed traits were milk, fat, protein, lactose, and milk energy yield, fat, protein, lactose, and milk energy content and somatic cell score. The lysine variant of the *DGATI* *K232A* mutation showed significant effects for most of the milk production traits. A specific allele of the *DGATI* promoter VNTR showed significant effects on the traits lactose yield and content, milk energy content, and somatic cell score compared to the other alleles. Additionally, a regulation mechanism between the *DGATI* *K232A* mutation and the *DGATI* promoter VNTR was found for fat yield and content, which could be caused by an upper physiological boundary for the effects of the *DGATI* gene. At the *CSN1S1* promoter 2 alleles out of 4 showed significant allele substitution effects on the milk yield traits.

(Key words: *DGATI*, casein promoter, daughter design, dairy cattle)

Abbreviation key: *CSN1S1* = α_{s1} -casein encoding gene, *DGATI* = acyl-CoA:diacylglycerol acyltransferase1, *K232A* = lysine to alanine substitution at position 232, VNTR = variable number of tandem repeats, YD = yield deviations

1. INTRODUCTION

In the last decade, different QTL for milk production traits have been mapped in various dairy cattle populations (reviewed by Bovenhuis and Schrooten, 2002; Khatkar et al., 2004). A segregating QTL with strong effects for milk production traits, especially for fat content in the centromeric region of bovine chromosome 14 (BTA14) was fine-mapped to a 3-cM region by Riquet et al. (1999) and later by Farnir et al. (2002). Subsequent studies identified a nonconservative dinucleotide substitution (*K232A*) in the acyl-CoA:diacylglycerol acyltransferase1 (*DGATI*) gene at positions 10433 and 10434 in exon number VIII as the most likely mechanism underlying the QTL on this chromosome (Grisart et al., 2002; Winter et al., 2002). The *DGATI* gene encodes the DGAT1 enzyme, which catalyses the final step of the triglyceride synthesis. The DGAT1 lysine variant increases fat and protein content, as well as fat yield, whereas the DGAT1 alanine variant increases milk and protein yield (e.g., Grisart et al., 2002; Winter et al., 2002; Thaller et al., 2003). In an expression study, it was shown that there is a small difference in the expression level of mRNA derived from bovine mammary gland tissue for the 2 *DGATI* variants, which is due to the structural mutation at *K232A* (Grisart et al., 2004). A difference in the enzyme activity level (measured as V_{\max}) in producing triglycerides was observed between the 2 variants, with V_{\max} being higher for the lysine variant (Grisart et al., 2004).

In subsequent studies, at least one additional source of variation besides the diallelic *DGATI* *K232A* mutation was postulated to be responsible for the QTL in the centromeric region at BTA14 (Winter et al., 2002; Bennewitz et al., 2004). In the German Holstein population, Kühn et al. (2004) described 5 alleles at a variable number of tandem repeat (VNTR) polymorphism in the *DGATI* promoter, which showed an additional effect on fat content next to the *DGATI* *K232A* mutation.

Besides the centromeric region of BTA14, the casein cluster on BTA6 is a genomic region where several QTL have been postulated for milk production traits. Some studies investigated the effects of the casein cluster on BTA6 with special emphasis on protein content in different dairy cattle breeds (Velmala et al., 1999; Ron et al., 2001; Olsen et al., 2005). Prinzenberg et al. (2003) observed associations between milk production traits and *CSN1S1* (α_{s1} -casein encoding gene) variants in the 5'-flanking region in the German Holstein population. The authors reported a superior effect of allele 4 compared to all other alleles. In a recent study, Kuss et al. (2005) reported an A to G exchange at position -175 bp in the promoter region of the bovine α_{s1} -casein encoding gene. Animals carrying the G variant showed higher milk content traits and a higher quantity of α_{s1} -casein compared to the animals carrying the A

variant. The authors argued that the G variant might be involved in the regulation mechanism of the *CSN1S1* expression.

The main objective of this study was the characterisation of the evidence, of the frequencies and of the effects of the alleles at the *DGAT1 K232A* mutation, the *DGAT1* promoter VNTR, and the *CSN1S1* promoter in the German Angeln population. The Angeln breed is located in the North of Germany. For several years, this breed has been crossed with different red breeds such as Red Holstein, Swedish Red and White, and Finnish Ayrshire (Savaş et al., 1998), may resulting in a higher genetic variability compared to e.g., Holstein Friesian. Among others, the characteristics of this breed are high milk protein and fat contents. Next to the traditional milk production traits, i.e., milk, fat, and protein yield, fat and protein content as well as somatic cell score, the traits lactose yield and content and milk energy yield and content were included in the analysis.

2. MATERIAL AND METHODS

2.1 Pedigree information

Following the daughter design (Weller et al., 1990), 5 families with a total of 805 daughters were selected for the genotyping process because of their relatively large number of available female half-sibs. The family size ranged between 123 and 199 daughters per sire with an average of 161 daughters. For the genotyping process, blood samples of the daughters and semen samples of the sires were used. No blood samples were available from the daughters' dams. A marker-assisted estimation of pedigree errors revealed 7% conflicting daughters (i.e., genotypes of daughters and of respective sires did not match) (see chapter one of this thesis). These daughters were excluded from the analysis, and therefore, the total number of daughters included was 749.

2.2 Genotyping data

The 5 families were genotyped for the *DGAT1 K232A* mutation, the *DGAT1* promoter VNTR, and the *CSN1S1* promoter. The genotyping of *DGAT1 K232A* was carried out by a PCR-RFLP test based on the *K232A* substitution at *DGAT1 K232A*. The following primers were used for the amplification of a 222-bp PCR fragment containing the *DGAT1 K232A* mutation (at position 10433/10434 in exon number VIII) of the sequence AY065621 (similar to the positions and numbers of the GenBank; Grisart et al., 2002): *DGAT1*_{6994L22} 5'-GCGGGGGAAGTTGAGCTCGTAG-3' and *DGAT1*_{6785U30} 5'-CCTGACTGGCCGCCTGC

CGCTTGCTCGTAG-3'. The 15- μ l PCR reaction consisted of 5 μ l of 20 ng genomic DNA, 2 pmol of each primer, 1.5 mM MgCl₂, 0.2 mM dNTPs, and 0.4 units recombinant *Taq* polymerase (Invitrogen GmbH, Karlsruhe, Germany). The PCR fragment was digested by the restriction enzyme *EaeI* (*CfrI*) (Amersham Pharmacia Biotech, Little Chalfont, UK) and was analysed using the MegaBACE 500 Analysis System (Amersham Biosciences Europe GmbH, Freiburg, Germany). The uncut fragment (222 bp) presented the lysine variant (K232) and the cut fragment (184 bp) the alanine variant (232A). The lysine variant was denoted as the K variant and the alanine variant as the A variant.

The amplification of the *DGATI* promoter VNTR was conducted in a different way as described by Kühn et al. (2004) because the primers used by Kühn et al. (2004) did not amplify in our laboratory. One reason could be the GC-rich sequences of the primer pair used by Kühn et al. (2004) which were located in a region between 1439 to 1565 at sequence AJ318490. The amplification of a *DGATI* promoter VNTR fragment comprised the repeat region between 1421 to 1666 of the *DGATI* sequence AJ318490 (similar to the positions and numbers of GenBank; Winter et al., 2002). PCR was carried out with the following primers: *DGATI*_{1421U21} 5'-ACCCTGGCAGCACCTCAATCA-3' and *DGATI*_{1643L24} 5'-CAATGAGAA GGCACGGACTGTGAA-3'. The primers were designed by using the Primer3 programme (Rozen and Skaletsky, 2000). The characteristic of these primers is a high T_m (melting point), taking the GC-rich template into account. The 10- μ l PCR reaction consisted of 3 μ l of 20 ng genomic DNA, 3 pmol of each primer, 1 mM MgCl₂, 0.3 mM dNTP's, and 0.125 units Platinum *Pfx* DNA Polymerase (Invitrogen GmbH, Karlsruhe, Germany). This polymerase works with a PCR_X Enhancer System which facilitates efficient amplification of GC-rich sequences. The electrophoresis was carried out by using the ABI377 (Applied Biosystems, Darmstadt, Germany). At the *DGATI* promoter VNTR, 6 alleles were found, which were denoted as VNTR alleles A, B, C, D, E, and F, respectively. VNTR allele F was only present in 2 unrelated daughters and was excluded from the statistical analysis. The genotyping of the *CSN1S1* promoter was carried out as described by Prinzenberg et al. (2003), and the alleles at this promoter were denoted in accordance with that study. Genotypes were stored in the ADRDB database (Reinsch, 1999) and were checked for agreement with the Mendelian laws of inheritance using the programme GENCHECK (Bennewitz et al., 2002).

2.3 Phenotypic data

Yield deviations (YD) (Wiggans and VanRaden, 1991) were used as the phenotypic units of measurement. For the traits milk yield, fat yield, protein yield, and somatic cell score, YD were taken from the August 2004 routine national breeding evaluation for the Angeln breed (VIT, Verden, Germany). No YD were calculated in the routine national breeding evaluation for fat and protein content, milk energy yield and content, as well as lactose yield and content. Therefore, YD for fat and protein content were calculated by using the following formula (in accordance with VIT, 2003), illustrated for protein content:

$$YD(\text{protein content}) = \frac{YD(\text{protein yield}) \times 100 - YD(\text{milk yield}) \times PM(\text{protein content})}{YD(\text{milk yield}) + PM(\text{milk yield})},$$

where PM is the population mean. For those traits, that are not routinely evaluated, REML-estimates for genetic parameters were first estimated by using a fixed-regression test-day model (Reinsch and Bennewitz, unpublished), and these estimates were subsequently employed in order to obtain YD for lactose yield and content as well as milk energy yield and content. The YD for the first three lactations were evaluated as a weighted average (see Remark A).

2.4 Statistical analyses regarding *DGATI*

Allele frequencies of *DGATI K232A* and the *DGATI* promoter VNTR were estimated using a maximum-likelihood procedure (for more details see chapter one of this thesis).

The statistical analyses were carried out using a weighted regression approach. Weights for the observations were the reciprocal of the variance of the YD (see Remark A).

The allele substitution effects of the K variant at *DGATI K232A* were estimated as follows:

$$y_{ij} = s_i + b_1 \times x_{ij} + e_{ij}, \quad (1)$$

with y_{ij} the phenotype of the j th daughter of the i th sire, s_i the fixed effect of the i th sire, x_{ij} the number of copies (0, 1, 2) of the K variant of the j th daughter of the i th sire, and e_{ij} the random residual effect. Because of the daughter design, the regression coefficient b_1 represents the allele substitution effect of the K variant.

Additionally, it was possible to estimate putative dominance effects for the genotypes of *DGATI K232A* because of the used daughter design (Falconer and Mackay, 1996). The dominance effects were estimated by treating the number of copies (0, 1, 2) of the K variant as classification variables in model (1). It was tested, whether the LS-means of the heterozygous animals were midway between the homozygous animals.

The allele substitution effects of the different alleles (A-E) at the *DGATI* promoter VNTR were estimated by multiple regression on the number of copies of the K variant at *DGATI K232A* and on the number of copies of the respective VNTR allele:

$$y_{ij} = s_i + b_1 \times x_{ij} + b_2 \times z_{A,ij} + b_3 \times z_{B,ij} + b_4 \times z_{C,ij} + b_5 \times z_{D,ij} + b_6 \times z_{E,ij} + e_{ij}, \quad (2)$$

where $z_{A-E,ij}$ is the number of copies (0, 1, 2) of the respective allele (A-E) at the *DGATI* promoter VNTR of the j th daughter of the i th sire and b_n is the respective regression coefficient. To avoid dependencies in the coefficient matrix, the effect of the VNTR allele E was set to zero. This model resulted in significant effects of the VNTR allele E compared to the other alleles and for that reason, its effect was re-estimated by applying the following simplified model:

$$y_{ij} = s_i + b_1 \times x_{ij} + b_E \times z_{E,ij} + e_{ij}, \quad (3)$$

with b_E the regression coefficient presenting the substitution effect of the VNTR allele E compared to all other alleles (Falconer and Mackay, 1996).

The haplotypes of *DGATI K232A* and of the *DGATI* promoter VNTR of the 5 sires were derived from the genotypes of their respective daughters. Subsequently, the haplotypes of the daughters were determined using the haplotypes of their sires, under the assumption that no recombination occurs between *DGATI K232A* and the *DGATI* promoter VNTR. The different haplotypes were denoted as KO, KE, AO, and AE, where O presents all other alleles at the *DGATI* promoter VNTR, excluding allele E (Table 2). Maximum-likelihood estimations of haplotype frequencies were carried out as described in chapter one of this thesis. To include daughters whose haplotypes cannot be unequivocally derived, an estimation of the posterior probability for the possible haplotypes was carried out (Remark B). The substitution effects of the different haplotypes were estimated using the following model:

$$y_{ij} = s_i + b_{xz} \times h_{xz,ij} + e_{ij}, \quad (4)$$

with s_i as the fixed effect of the sire i , b_{xz} the respective regression coefficient, and $h_{xz,ij}$ the number of copies (0, 1, 2) of the respective haplotype of the j th daughter of the i th sire. The indices x and z represent the respective alleles of the *DGATI K232A* mutation and of the *DGATI* promoter VNTR. Model (4) was applied for all 4 haplotypes in turn. In the case of not unequivocally derived haplotypes the corresponding posterior probabilities were used as the regression variables. To test whether the effects of the haplotypes are additive, model (4) was applied, but the number of copies of the haplotypes was treated as a classification variable. Therefore, only the unequivocally derived haplotypes were used. The comparison of the respective LS-means of the haplotype classes reveal a putative non-additivity.

Similarly, the existence of a putative interaction between the *DGATI K232A* mutation and the *DGATI* promoter VNTR was determined by:

$$y_{ij} = s_i + b_1 \times x_{ij} + b_E \times z_{E,ij} + b_{xz} \times h_{xz,ij} + e_{ij}. \quad (5)$$

The regression variable $h_{xz,ij}$ represents a putative interaction. For xz all 4 haplotypes were used in turn. In a next step, a putative interaction between the haplotypes was tested using the following model:

$$y_{ij} = s_i + h_{xz,ij} + b_{x'z'} \times h_{x'z',ij} + b_{x'z'} \times \omega_{xzx'z',ij} + e_{ij}, \quad (6)$$

where $h_{xz,ij}$ is the fixed effect of the haplotype xz (i.e., the number of copies of the haplotype is modelled as a classification variable) and $\omega_{xzx'z',ij}$ is the interaction of the haplotypes xz and $x'z'$. This model tested whether the regression coefficient $b_{x'z'}$ of the haplotype $x'z'$ depended on the number of copies (either 0 or 1) of the haplotype xz carried by a daughter. All possible haplotype combinations were tested in turn ($x'z' \neq xz$).

2.5 Statistical analyses regarding *CSN1S1*

The allele frequencies of the *CSN1S1* promoter were estimated with the same maximum-likelihood function as the *DGATI* mutations.

A multiple weighted regression model was applied. Because of the highly significant effect of *DGATI K232A*, the model estimated the allele substitution effect of the different alleles (1-4) at the *CSN1S1* promoter by simultaneous regression on the number of copies (0, 1, 2) of the alleles at the *CSN1S1* promoter and on the number of copies of the K variant at *DGATI K232A*. This analysis was carried out twice; at first the effects of the *CSN1S1* allele 2 were set to zero and afterwards the effects of the *CSN1S1* allele 3 were set to zero. The following model was applied:

$$y_{ij} = s_i + b_1 \times x_{ij} + b_{v_1} \times v_{1,ij} + b_{v_2} \times v_{2,ij} + b_{v_3} \times v_{3,ij} + b_{v_4} \times v_{4,ij} + e_{ij}, \quad (7)$$

where b_{v_n} is the respective regression coefficient of the respective allele at the *CSN1S1* promoter and $v_{n,ij}$ is the number of copies (0, 1, 2) of the respective allele at the *CSN1S1* promoter. The remaining terms are the same as in model (1). The results of this model revealed a significant effect of the *CSN1S1* alleles 2 and 3 for the different milk production traits. Therefore, the following simplified model was applied:

$$y_{ij} = s_i + b_1 \times x_{ij} + b_{v_2} \times v_{2,ij} + e_{ij}. \quad (8)$$

The regression coefficient b_{v_2} presents the allele substitution effect of the *CSN1S1* allele 2 compared to all other alleles. The same model was applied for the *CSN1S1* allele 3.

3. RESULTS

3.1 Allele frequencies and haplotype frequencies

Table 1 presents the allele frequencies of the *DGATI* K232A mutation, of the *DGATI* promoter VNTR, and of the *CSN1S1* promoter. The allele frequency of the K variant at *DGATI* K232A was 0.61. Three sires were heterozygous at *DGATI* K232A and 2 were homozygous for the K variant. The most frequent *DGATI* promoter VNTR allele in the Angeln population was the VNTR allele E (0.38). In the case of the *CSN1S1* promoter, 4 alleles were segregating in the Angeln dairy cattle population, which is in accordance with Prinzenberg et al. (2003). The most frequent *CSN1S1* allele in this population was the allele 2. Three sires were homozygous for this allele.

Table 1: Allele frequencies of the *DGATI* K232A, of the *DGATI* promoter VNTR, and of the *CSN1S1* promoter

Allele ¹	<i>DGATI</i> K232A	Allele	<i>DGATI</i> promoter VNTR	Allele	<i>CSN1S1</i> promoter
K	0.61	A	0.01	1	0.01
A	0.39	B	0.25	2	0.70
		C	0.30	3	0.20
		D	0.06	4	0.09
		E	0.38		
		F	< 0.01		

¹ K denotes for lysine variant and A for alanine variant.

The frequencies of the haplotypes of the *DGATI* K232A and of the *DGATI* promoter VNTR are listed in Table 2. The most frequent haplotypes were the haplotypes KE and AO (0.38), whereas the frequency of the haplotype AE was very low (0.01).

Table 2: Haplotype frequencies of *DGATI K232A* and of the *DGATI* promoter VNTR

Haplotypes <i>DGATI K232A</i> and <i>DGATI</i> promoter VNTR ¹	Haplotype abbreviations as used in the text ¹	Frequencies
K232-O	KO	0.23
K232-E	KE	0.38
232A-O	AO	0.38
232A-E	AE	0.01

¹ All VNTR alleles except VNTR allele E were combined into one group (denoted as O).

3.2 Allele substitution effects of *DGATI K232A* and of the *DGATI* promoter VNTR

Average allele substitution effects of the K variant at *DGATI K232A* for the 10 milk production traits are shown in Table 3 (results of model (1)). For the content traits protein, fat, and milk energy content, as well as for fat yield, positive effects of the K variant were observed. The K variant showed stronger allele substitution effects for fat and milk energy content than for protein content. Negative effects were observed for the yield traits milk, protein, and lactose yield. The allele substitution effects of the K variant for milk ($\alpha = -87.69$ kg, $P < 0.001$), fat ($\alpha = 3.21$ kg, $P < 0.001$) and protein yield ($\alpha = -1.31$ kg, $P = 0.016$) were expected because of the results of previous studies (e.g., Spelman et al., 2002; Thaller et al., 2003; Bennewitz et al., 2004). The estimation of the dominance effects of the *DGATI K232A* alleles for the traits milk yield, lactose yield, milk energy content, and somatic cell score showed that the LS-means of the heterozygous genotypes (AK) were between the 2 homozygotes, indicating that *DGATI K232A* does not show any dominance effects (not shown).

Table 3: Allele substitution effects (α) of the K variant at *DGATI K232A* with standard errors (SE), F-value, and P-value for the different milk production traits, results from model (1)

Trait ¹	α	SE	F-value	P-value
MY (kg)	-87.69	15.83	30.68	< 0.001
PY (kg)	-1.31	0.54	5.78	0.016
PC (%)	0.03	0.004	59.23	< 0.001
FY (kg)	3.21	0.84	14.51	< 0.001
FC (%)	0.12	0.009	177.64	< 0.001
LY (g)	-9.54	2.10	20.59	< 0.001
LC (%)	0.005	0.003	2.88	0.090
EY (ME)	0.13	0.14	0.96	0.327
EC (ME/kg)	0.09	0.006	248.89	< 0.001
SCS	-0.009	0.01	0.78	0.376

¹ The abbreviations are: MY = milk yield; PY = protein yield; PC = protein content; FY = fat yield; FC = fat content; LY = lactose yield; LC = lactose content; EY = milk energy yield; EC = milk energy content; SCS = somatic cell score.

Table 4 presents the allele substitution effects of the K variant at *DGATI K232A* and of the *DGATI* promoter VNTR allele E for the 10 milk production traits (results of model (3)). For the traits lactose content and somatic cell score the VNTR allele E and the K variant showed significant effects in the opposite direction. For the traits lactose yield and milk energy content both, the K variant and the VNTR allele E showed significant negative effects and significant positive effects, respectively. For the traits milk yield, protein content, fat yield, and fat content only the K variant showed significant effects.

Table 4: Allele substitution effects of the K variant at *DGATI K232A* and of the *DGATI* promoter VNTR allele E on the milk production traits, with standard error (SE), and P-value, results from model (3)

Trait ¹	K variant			VNTR allele E		
	α	SE	P-value	α	SE	P-value
MY (kg)	-77.26	20.17	< 0.001	-20.74	20.29	0.31
PY (kg)	-0.98	0.69	0.155	-0.68	0.70	0.33
PC (%)	0.03	0.005	< 0.001	0.002	0.005	0.75
FY (kg)	3.59	1.07	< 0.001	-0.71	1.08	0.52
FC (%)	0.12	0.01	< 0.001	0.007	0.01	0.54
LY (g)	-6.46	2.66	0.015	-5.53	2.67	0.04
LC (%)	0.009	0.003	0.007	-0.008	0.004	0.03
EY (ME)	0.30	0.17	0.088	-0.29	0.17	0.10
EC (ME/kg)	0.08	0.007	< 0.001	0.01	0.007	0.07
SCS	-0.03	0.01	0.038	0.03	0.01	0.04

¹ For abbreviations see Table 3.

The substitution effects of the different haplotypes of *DGATI K232A* and the *DGATI* promoter VNTR are presented in Table 5 (results of model (4)). The results of the haplotype regression model support the results of the previous models (Tables 3 and 4). The haplotype KE showed significant negative effects for milk, protein, and lactose yield and positive effects for protein, fat, and milk energy content as well as fat yield. For the same traits, the haplotype AO showed effects in the opposite direction. For both haplotypes no significant effects were observed for the traits milk energy yield, lactose content, and somatic cell score. The effects of the haplotype KO were mainly in the same direction as those of the haplotype KE. The haplotype AE showed the highest effects for the main part of the traits. Because of the low number of daughters showing this haplotype (and no homozygous ones), the effects are mainly not significant and the standard deviations of estimates are high (Table 5).

Table 5: Substitution effects (α), standard errors (SE), and error probabilities (P-value) of the different haplotypes of *DGATI K232A* and the *DGATI* promoter VNTR on 10 milk production traits, results from model (4)

Trait ¹	Haplotype KE			Haplotype AE			Haplotype KO			Haplotype AO		
	α	SE	P-value	α	SE	P-value	α	SE	P-value	α	SE	P-value
MY (kg)	-73.19	16.00	< 0.001	-103.30	100.77	0.31	-19.75	20.52	0.340	90.97	16.13	< 0.001
PY (kg)	-1.25	0.55	0.020	-7.12	3.38	0.04	-0.03	0.69	0.970	1.49	0.55	0.007
PC (%)	0.02	< 0.01	< 0.001	-0.05	0.03	0.03	0.01	0.01	0.040	-0.03	< 0.01	< 0.001
FY (kg)	1.65	0.85	0.050	-1.20	5.30	0.82	2.90	1.07	0.007	-3.55	0.86	< 0.001
FC (%)	0.09	0.01	< 0.001	0.07	0.06	0.28	0.06	0.01	< 0.001	-0.13	0.01	< 0.001
LY (g)	-9.85	2.08	< 0.001	-11.71	13.15	0.37	1.05	2.69	0.700	9.88	2.13	< 0.001
LC (%)	< 0.01	< 0.01	0.770	-0.02	0.02	0.33	0.01	< 0.01	0.110	< -0.01	< 0.01	0.150
EY (ME)	-0.11	0.14	0.440	-0.62	0.85	0.47	0.43	0.17	0.010	-0.15	0.14	0.280
EC (ME/kg)	0.07	0.01	< 0.001	-0.01	0.04	0.76	0.03	0.01	< 0.001	-0.09	0.01	< 0.001
SCS	< 0.01	0.01	0.950	0.12	0.07	0.08	-0.02	0.01	0.180	0.01	0.01	0.470

¹ For abbreviations see Table 3.

3.3 Interaction between *DGAT1* K232A and *DGAT1* promoter VNTR

The LS-means of the 3 haplotype classes for the haplotypes KE and AO (0, 1, or 2 copies of the respective haplotype) are shown in Table 6. For haplotype KE, differences between classes 0 and 1 are larger than the differences between 1 and 2, for the traits protein content, fat yield and content. On the other hand, for haplotype AO, differences between classes 1 and 2 are larger compared to the differences between 0 and 1. These results show the non-additivity of these haplotypes. In contrast, it was observed that the LS-means for the traits milk yield and lactose yield of haplotypes KE and AO for class 1 are approximately between the values of classes 0 and 2. For the other traits and other haplotypes, no clear pattern regarding putative additivity was observed (not shown). This might either be due to non-significant effects of the haplotype for the respective trait (Table 5) or due to the low haplotype frequency (Table 2) and hence, due to the strong effect of the second haplotype of a daughter in case of a heterozygosity or even in case of carrying no copies of the respective haplotype (haplotype classes 1 and 0, respectively).

Table 6: LS-means of the 3 haplotype classes (0, 1, 2) for the haplotypes KE and AO with standard error in brackets

Trait ¹	Haplotype KE			Haplotype AO		
	Haplotype class 0	Haplotype class 1	Haplotype class 2	Haplotype class 0	Haplotype class 1	Haplotype class 2
MY (kg)	-133.30 (16.29)	-202.65 (14.87)	-268.90 (31.74)	-237.41 (15.38)	-147.36 (15.68)	-66.53 (32.81)
PY (kg)	0.47 (0.55)	-0.25 (0.51)	-2.22 (1.08)	-1.25 (0.53)	0.79 (0.54)	0.71 (1.12)
PC (%)	0.09 (0.004)	0.11 (0.004)	0.12 (0.008)	0.12 (0.004)	0.10 (0.004)	0.05 (0.008)
FY (kg)	8.34 (0.87)	11.14 (0.79)	10.30 (1.70)	11.75 (0.82)	9.51 (0.83)	2.91 (1.75)
FC (%)	0.24 (0.01)	0.34 (0.009)	0.38 (0.02)	0.38 (0.009)	0.27 (0.009)	0.10 (0.02)
LY (g)	7.23 (2.13)	-0.09 (1.94)	-13.98 (4.13)	-4.80 (2.03)	5.19 (2.06)	13.54 (4.34)
LC (%)	-0.03 (0.003)	-0.04 (0.003)	-0.03 (0.005)	-0.03 (0.003)	-0.04 (0.003)	-0.04 (0.006)
EY (ME)	1.56 (0.14)	1.61 (0.13)	1.24 (0.27)	1.63 (0.13)	1.56 (0.14)	1.12 (0.28)
EC (ME/kg)	-0.005 (0.006)	0.06 (0.006)	0.13 (0.01)	0.10 (0.005)	0.01 (0.006)	-0.10 (0.01)
SCS	-0.03 (0.01)	-0.02 (0.01)	-0.03 (0.02)	-0.03 (0.01)	-0.03 (0.01)	-0.01 (0.01)

¹ For abbreviations see Table 3.

The results of model (5) revealed a statistically significant interaction effect between *DGATI* *K232A* and *DGATI* promoter VNTR for fat content, because all 3 regression variables were significant (in all cases $P < 0.02$), when haplotype KE was included in the model. The estimated regression coefficient for the haplotype KE was negative (not shown), indicating that the positive effects of the K variant and the VNTR allele E are smaller for daughters carrying copies of both alleles (Table 4).

A statistically significant interaction between the haplotypes KE and KO was detected for milk energy content ($P = 0.03$, results of model (6)). More specifically, the effect of the haplotype KE was only significant for those daughters that not carrying a copy of the haplotype KO ($P < 0.001$).

3.4 Allele substitution effects of the *CSN1S1* promoter

The multiple regression model (7) on the different number of copies of the alleles at the *CSN1S1* promoter indicated that the allele substitution effects of the alleles 2 and 3 on the yield traits milk, protein, fat, lactose, and milk energy yield, as well as milk energy content showed both statistically significant effects but in the opposite direction (Table 7). For example, the *CSN1S1* allele 2 showed an allele substitution effect of $\alpha = -0.37$ for the trait milk energy yield ($P = 0.02$), whereas the *CSN1S1* allele 3 affects this trait in a positive way ($\alpha = 0.56$; $P = 0.004$). Neither the *CSN1S1* allele 2 nor the *CSN1S1* allele 3 showed any significant effects for content traits or somatic cell score.

Table 7: Allele substitution effects (α), standard errors (SE), and P-values of the alleles 2 and 3 at the *CSN1S1* promoter compared to the other alleles on 10 milk production traits, results of model (8)

Trait ¹	<i>CSN1S1</i> allele 2			<i>CSN1S1</i> allele 3		
	α	SE	P-value	α	SE	P-value
MY (kg)	-35.85	18.38	0.05	51.10	22.31	0.020
PY (kg)	-1.21	0.63	0.06	1.95	0.77	0.010
PC (%)	< 0.01	0.01	0.71	< 0.01	0.01	0.800
FY (kg)	-1.92	0.98	0.05	2.10	1.18	0.080
FC (%)	< -0.01	0.01	0.95	-0.01	0.01	0.520
LY (g)	-6.38	2.45	0.01	9.52	2.97	0.001
LC (%)	< -0.01	< 0.01	0.44	< -0.01	0.01	0.660
EY (ME)	-0.37	0.16	0.02	0.56	0.19	0.004
EC (ME/kg)	0.01	0.01	0.30	-0.01	0.01	0.090
SCS	-0.01	0.01	0.33	0.02	0.02	0.230

¹ For abbreviations see Table 3.

4. DISCUSSION

4.1 Allele frequencies for *DGATI K232A*

The estimated allele frequency of 0.61 for the K variant at *DGATI K232A* in the German Angeln dairy cattle population is identical with the frequencies reported by Kaupe et al. (2004) for the same breed, but higher than the frequencies reported for Holstein Friesians. Thaller et al. (2003) reported an allele frequency in the German Holstein population of 0.55. Other studies in the Holstein population and in the Polish Black-and-White population estimated allele frequencies between 0.30 and 0.68 (Bovenhuis and Schrooten, 2002; Winter et al., 2002; Pareek et al., 2005). The K frequency in the German Fleckvieh population is substantially lower (0.07; Thaller et al., 2003). The relatively high frequency of the K variant in the German Angeln population could be due to the selection on content traits in former years, which is the same for the Jersey population. This is supported in the present study by the fact that 2 sires were homozygous for the K variant and that none of the sires was homozygous for the A variant. The population mean of the fat content in the Jersey breed is even higher compared to the German Angeln breed and the K frequency is also somewhat higher in the Jerseys (between 0.69 and 0.88; Spelman et al., 2002; Winter et al., 2002; Kaupe et al., 2004).

4.2 Allele substitution effects of *DGATI K232A* and of the *DGATI* promoter VNTR

The estimated allele substitution effects of *DGATI K232A* on the milk production traits in the German Angeln population were substantially lower than those in the German Holstein population, but they affect the different traits in the same direction (Table 3; Thaller et al., 2003; Bennewitz et al., 2004). Spelman et al. (2002) reported almost the same effects for the K variant in the New Zealand Jersey population as observed in the German Angeln population. One reason for lower allele substitution effects in the Angeln and the Jersey breeds could be that both breeds showed higher population means for the content traits. Additionally, the influence of the K variant did not affect the content traits on the same level as in the Holstein Friesian breed, because of a higher base level of the triglyceride synthesis in these breeds. The limiting factor for a higher triglyceride synthesis might be the limited availability of the 2 substrates diacylglycerol and fatty acyl-CoA in the final step of the triglyceride synthesis.

The K variant at *DGATI K232A* showed no significant effects for the milk energy yield but highly significant effects for the yield traits milk, protein, and fat. Hence, *DGATI K232A* behaves neutrally with regard to the milk energy yield. This neutral character is due to the redistribution of the milk energy of the cow between the 3 milk components fat, protein, and lactose depending on the corresponding *DGATI K232A* genotype.

It was observed that the VNTR allele E showed significant effects for some milk production effects compared to all other alleles at the *DGATI* promoter VNTR. These same results were reported by Kühn et al. (2004) for the *DGATI* VNTR allele 5. However, in contrast to Kühn et al. (2004), the VNTR allele E was mainly linked to the K variant at *DGATI K232A* (Table 2), whereas the *DGATI* VNTR allele 5 showed up with the A variant in the German Holstein Friesian population (Kühn et al., 2004). It is likely that the VNTR allele E corresponds to the *DGATI* VNTR allele 5 of Kühn et al. (2004), but it was not possible to verify this. An interesting point is the significance of *DGATI K232A* and the promoter VNTR for somatic cell score and lactose content (results of model (3), Table 4). This could not be observed for *DGATI K232A* without the promoter VNTR in the model (results of model (1), Table 3), probably because of the opposite direction of the K variant and the VNTR allele E and hence, of the neutral effect of haplotype KE on these traits (Table 5). Following this, both mutations also affect udder health, because both traits are well-known indicator traits for udder health in dairy cattle. Additionally, whereas the K variant and the VNTR allele E affect the milk production traits in the same direction, this is not the case for udder health.

For daughters with the haplotypes KE, AE, and KO, it was shown that the effects on the different milk production traits were in general in the same direction (results of Table 5). Compared to the haplotype KO, the haplotype KE showed substantially higher effects for the traits milk yield and protein yield and content. For example for milk yield, the effect of the haplotype KO was $\alpha = -19.75$ ($P = 0.34$) and of the haplotype KE the effect was $\alpha = -73.19$ ($P < 0.001$). The comparison of the effects of the haplotypes AE and AO emphasises the strong effect of the VNTR allele E. Despite the opposite effects of the A variant at *DGATI K232A* and the VNTR allele E, the effects of the haplotype AE were determined by the effects of the VNTR allele E. This is illustrated e.g., by the effects on protein yield, where the haplotype AO showed a positive effect ($\alpha = 1.49$, $P = 0.007$), whereas the haplotype AE showed a negative effect ($\alpha = -7.12$, $P = 0.04$). The consequence is, that daughters with the haplotype AE showed the same phenotype as daughters with the haplotype KE. It must be kept in mind that the presented results, especially for the haplotype AE, are not always significant because of the low frequency of this haplotype (Table 2).

However, the effects of the *DGATI K232A* and the *DGATI* promoter VNTR did not act strictly additively. This becomes obvious by comparing the effects of the mutations separately (Table 4) and combined (Table 5), and additionally the LS-means in Table 6. For example, the effects of the K variant and of the VNTR allele E on fat content were $\alpha = 0.12$ and $\alpha = 0.007$, respectively (Table 4), but the effect of the haplotype KE was not the sum of both but only $\alpha = 0.09$. Additionally, the effects of the haplotypes KE, AO, and KO were on a similar level for this trait (Table 5). Hence, it seems that there is an upper physiological boundary for the effect, especially for fat content, due to the haplotypes, and this limit is reached by both the K variant and the VNTR allele E, regardless of the allele of the respective other mutation (*DGATI K232A* and promoter VNTR, respectively). The non-strict additive behaviour of these 2 mutations for fat content was also formally shown by the statistical significance of the interaction test (results of model (5)) and by the results of the haplotype interaction model for milk energy content (results of model (6)).

4.3 Effects on BTA6 affected by the *CSN1S1* promoter

The allele frequencies at the *CSN1S1* promoter are on a similar level as reported in the German Holsteins (Table 1, Prinzenberg et al., 2003). Opposite results to those reported by Prinzenberg et al. (2003) were observed for the substitution effects of the included alleles at the *CSN1S1* promoter. In the Angeln population, significant allele substitution effects were found for the yield traits, either for *CSN1S1* alleles 2 or 3, whereas no significant effects were

observed for the content traits and somatic cell score. Prinzenberg et al. (2003) found only significant allele substitution effects for protein content and suggested that the *CSN1S1* allele 4 affects this trait in a positive way. This effect was not confirmed in the present study. This discrepancy could be due to the small number of daughters carrying this allele in the Angeln population (low frequency of *CSN1S1* allele 4, Table 1). On the basis of the study of Prinzenberg et al. (2003) and the present study it remains to be investigated if the *CSN1S1* promoter could be a functional candidate locus for the trait protein content as discussed by Prinzenberg et al. (2003). Another hypothesis is that the observed effects are due to a linkage disequilibrium between the *CSN1S1* promoter and the causative mutation.

5. CONCLUSIONS

The allele substitution effects on milk production traits of the *DGATI K232A* mutation, the *DGATI* promoter VNTR, the respective *DGATI* haplotypes, and the *CSN1S1* promoter were investigated in this study. Segregation of 6 alleles at the *DGATI* promoter VNTR and of 4 alleles at the *CSN1S1* promoter was found in the Angeln population. Besides *DGATI K232A*, one allele of the *DGATI* promoter VNTR showed significant effects on the traits lactose yield and content, milk energy content, and somatic cell score. No dominance effect for *DGATI K232A* was observed. A non-strict additivity of the effects of *DGATI K232A* and *DGATI* promoter VNTR haplotypes was found, probably due to an upper physiological limit of the effects for fat yield and content due to the *DGATI* gene. Furthermore, significant allele substitution effects were observed for 2 alleles at the *CSN1S1* promoter for the milk yield traits.

APPENDIX

Remark A. Estimation of the variance of the yield deviations

The yield deviations for the first three lactations were used as phenotypes. They were combined into one value ($YD_{1-3.lactation}$) by weighting the three yield deviations as follows:

$$YD_{1-3.lactation} = \mathbf{w}'\mathbf{y},$$

with \mathbf{w}' being a vector of the weights for the three lactations, i.e., $\mathbf{w}' = [w_1 \quad w_2 \quad w_3]$, and \mathbf{y} being a vector with the yield deviations of the corresponding lactation, i.e., $\mathbf{y}' = [YD_1 \quad YD_2 \quad YD_3]$. The values w_1 to w_3 were calculated as the number of the test milkings in the respective lactation (n_1 , n_2 , and n_3 , respectively) divided by the total number of test milkings (i.e., $n_1 + n_2 + n_3$). The variances of the $YD_{1-3.lactation}$ were estimated as:

$$\text{Var}(YD_{1-3.\text{lactation}}) = \mathbf{w}' \mathbf{V} \mathbf{w},$$

\mathbf{V} represents the variance-covariance matrix of the yield deviation in the three lactations. This matrix can be written as:

$$\mathbf{V} = \text{Var} \begin{bmatrix} YD_1 \\ YD_2 \\ YD_3 \end{bmatrix} = \begin{bmatrix} (\sigma_{a1}^2 + \sigma_{p1}^2 + \frac{1}{n_1} \sigma_{e1}^2) & (\sigma_{a1,a2} + \sigma_{p1,p2}) & (\sigma_{a1,a3} + \sigma_{p1,p3}) \\ (\sigma_{a1,a2} + \sigma_{p1,p2}) & (\sigma_{a2}^2 + \sigma_{p2}^2 + \frac{1}{n_2} \sigma_{e2}^2) & (\sigma_{a2,a3} + \sigma_{p2,p3}) \\ (\sigma_{a1,a3} + \sigma_{p1,p3}) & (\sigma_{a2,a3} + \sigma_{p2,p3}) & (\sigma_{a3}^2 + \sigma_{p3}^2 + \frac{1}{n_3} \sigma_{e3}^2) \end{bmatrix},$$

with σ_{aj}^2 , σ_{pj}^2 , and σ_{ej}^2 being the additive genetic, permanent environment, and residual variance of lactation j , and $\sigma_{aj,aj'}$ and $\sigma_{pj,pj'}$ being the covariance of the additive genetic and permanent environment variance of lactation j and j' ($j \neq j'$). The variance components were taken from Reinsch and Bennewitz (unpublished).

Remark B. Calculation of the posterior probability

Posterior probabilities for the haplotypes were used in the statistical analysis for daughters, whose haplotypes of the *DGAT1 K232A* mutation and the *DGAT1* promoter VNTR could not be unequivocally derived. The calculation of the posterior probability will be demonstrated by an example: The haplotype of *DGAT1 K232A* and the *DGAT1* promoter VNTR of a sire was KC and KD. The 2 possibilities for the haplotypes of a daughter, if the daughter has the genotypes AK for *DGAT1 K232A* and CD for the *DGAT1* promoter VNTR, will be AC and KD (first possibility) or AD and KC (second possibility). In the first (second) case the daughters inherited the haplotype AC (AD) from the population. The posterior probability for the first possibility is:

$$p_1 = \frac{0.5f(\text{AC})}{(0.5f(\text{AC})) + (0.5f(\text{AD}))},$$

with $f(\text{AC})$ and $f(\text{AD})$ the frequencies of the haplotypes AC and AD, respectively. The posterior probability for the second possibility is: $p_2 = 1 - p_1$.

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REFERENCES

- Bennewitz, J., N. Reinsch, and E. Kalm. 2002. GENCHECK: A program for consistency checking and derivation of genotypes at co-dominant and dominant loci. *J. Anim. Breed. Genet.* 119(5):350–360.
- Bennewitz, J., N. Reinsch, S. Paul, C. Looft, B. Kaupe, C. Weimann, G. Erhardt, G. Thaller, Ch. Kühn, M. Schwerin, H. Thomsen, F. Reinhardt, R. Reents, and E. Kalm. 2004. The DGAT1 K232A mutation is not solely responsible for the milk production quantitative trait locus on the bovine chromosome 14. *J. Dairy Sci.* 87(2):431–442.
- Bovenhuis, H., and C. Schrooten. 2002. Quantitative trait loci for milk production traits in dairy cattle. *Proc. 7th World Congr. Genet. Appl. Livestock Prod.* No. 09–07, Montpellier, France. Vol. 31:27–34.
- Falconer, D. S., and T. F. C. Mackay. 1996. *Introduction to Quantitative Genetics*. 4th ed. Longman Scientific and Technical, New York, NY.
- Farnir, F., B. Grisart, W. Coppieters, J. Riquet, P. Berzi, N. Cambisano, L. Karim, M. Mni, S. Moisisio, P. Simon, D. Wagenaar, J. Vilkki, and M. Georges. 2002. Simultaneous mining of linkage and linkage disequilibrium to fine map quantitative trait loci in outbred half-sib pedigrees: Revisiting the location of a quantitative trait locus with major effect on milk production on bovine chromosome 14. *Genetics* 161(1):275–287.
- Grisart, B., W. Coppieters, F. Farnir, L. Karim, C. Ford, P. Berzi, N. Cambisano, M. Mni, S. Reid, P. Simon, R. Spelman, M. Georges, and R. Snell. 2002. Positional candidate cloning of a QTL in dairy cattle: Identification of a missense mutation in the bovine DGAT1 gene with major effect on milk yield and composition. *Genome Res.* 12(2):222–231.
- Grisart, B., F. Farnir, L. Karim, N. Cambisano, J.-J. Kim, A. Kvasz, M. Mni, P. Simon, J.-M. Frère, W. Coppieters, and M. Georges. 2004. Genetic and functional confirmation of the causality of the *DGAT1* K232A quantitative trait nucleotide in affecting milk yield and composition. *Proc. Natl. Acad. Sci. USA* 101(8):2398–2403.
- Kaupe, B., A. Winter, R. Fries, and G. Erhardt. 2004. *DGAT1* polymorphism in *Bos indicus* and *Bos taurus* cattle breeds. *J. Dairy Res.* 71(2):182–187.

- Khatkar, M. S., P. C. Thomson, I. Tammen, and H. W. Raadsma. 2004. Quantitative trait loci mapping in dairy cattle: review and meta-analysis. *Genet. Sel. Evol.* 36:163–190.
- Kühn, Ch., G. Thaller, A. Winter, O. R. P. Bininda-Emonds, B. Kaupe, G. Erhardt, J. Bennewitz, M. Schwerin, and R. Fries. 2004. Evidence for multiple alleles at the *DGATI* locus better explains a quantitative trait locus with major effect on milk fat content in cattle. *Genetics* 167(4):1873–1881.
- Kuss, A. W., J. Gogol, H. Bartenschlager, and H. Geldermann. 2005. Polymorphic AP-1 binding site in bovine *CSN1S1* shows quantitative differences in protein binding associated with milk protein expression. *J. Dairy Sci.* 88(6):2246–2252.
- Olsen, H. G., S. Lien, M. Gautier, H. Nilsen, A. Roseth, P. R. Berg, K. K. Sundaasen, M. Svendsen, and T. H. E. Meuwissen. 2005. Mapping of a milk production quantitative trait locus to a 420-kb region on bovine chromosome 6. *Genetics* 169(1):275–283.
- Pareek, C. S., U. Czarnik, T. Zabolewicz, R. S. Pareek, and K. Walawski. 2005. *DGATI* *K232A* quantitative trait nucleotide polymorphism in Polish Black-and-White cattle. *J. Appl. Genet.* 46(1):85–87.
- Prinzenberg, E.-M., C. Weimann, H. Brandt, J. Bennewitz, E. Kalm, M. Schwerin, and G. Erhardt. 2003. Polymorphism of the bovine *CSN1S1* promoter: linkage mapping, intragenic haplotypes, and effects on milk production traits. *J. Dairy Sci.* 86(8):2696–2705.
- Reinsch, N. 1999. A multiple-species, multiple-project database for genotypes at codominant loci. *J. Anim. Breed. Genet.* 116(5):425–435.
- Riquet, J., W. Coppieters, N. Cambisano, J.-J. Arranz, P. Berzi, S. K. Davis, B. Grisart, F. Farnir, L. Karim, M. Mni, P. Simon, J. F. Taylor, P. Vanmanshoven, D. Wagenaar, J. E. Womack, and M. Georges. 1999. Fine-mapping of quantitative trait loci by identity by descent in outbred populations: Application to milk production in dairy cattle. *Proc. Natl. Acad. Sci. USA* 96(16):9252–9257.
- Ron, M., D. Kliger, E. Feldmesser, E. Seroussi, E. Ezra, and J. I. Weller. 2001. Multiple quantitative trait locus analysis of bovine chromosome 6 in the Israeli Holstein population by a daughter design. *Genetics* 159(2):727–735.
- Rozen, S., and H. S. Skaletsky. 2000. Primer3 on the www for general users and for biologist programmers. In: Krawetz S., Misener S. (eds.) *Bioinformatics Methods and Protocols: Methods in Molecular Biology*. Humana Press, Totowa, NJ, pp 365–386. http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi.

- Savaş, T., N. Reinsch, and E. Kalm. 1998. Auswirkungen der Rassenzusammensetzung auf Merkmale der Tagesmilchmenge und auf die Zellzahl beim Angler Rind. *Arch. Tierz.* 41(3):201–209.
- Spelman, R. J., C. A. Ford, P. McElhinney, G. C. Gregory, and R. G. Snell. 2002. Characterization of the *DGAT1* gene in the New Zealand dairy population. *J. Dairy Sci.* 85(12):3514–3517.
- Thaller, G., W. Krämer, A. Winter, B. Kaupe, G. Erhardt, and R. Fries. 2003. Effects of *DGAT1* variants on milk production traits in German cattle breeds. *J. Anim. Sci.* 81(8):1911–1918.
- Velmala, R. J., H. J. Vilkki, K. T. Elo, D. J. de Koning, and A. V. Mäki-Tanila. 1999. A search for quantitative trait loci for milk production traits on chromosome 6 in Finnish Ayrshire cattle. *Anim. Genet.* 30(2):136–143.
- VIT. 2003. Zuchtwertschätzung, Vereinigte Informationssysteme Tierhaltung w.V., Verden/Aller, Germany. <http://www.vit.de>.
- Weller, J. I., Y. Kashi, and M. Soller. 1990. Power of daughter and granddaughter designs for determining linkage between marker loci and quantitative trait loci in dairy cattle. *J. Dairy Sci.* 73(9):2525–2537.
- Wiggans G. R. and P. M. VanRaden. 1991. Method and Effect of Adjustment for Heterogeneous Variance. *J. Dairy Sci.* 74(12): 4350–4357.
- Winter, A., W. Krämer, F. A. O. Werner, S. Kollers, S. Kata, G. Durstewitz, J. Buitkamp, J. E. Womack, G. Thaller, and R. Fries. 2002. Association of a lysine-232/alanine polymorphism in a bovine gene encoding acyl-CoA:diacylglycerol acyltransferase (*DGAT1*) with variation at a quantitative trait locus for milk fat content. *Proc. Natl. Acad. Sci. USA* 99(14):9300–9305.

CHAPTER THREE

QTL mapping for ten milk production traits in the German Angeln dairy cattle population

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ABSTRACT

A mapping study for quantitative trait loci (QTL) affecting ten milk production traits (milk, fat, protein, lactose, and milk energy yield, fat, protein, lactose, and milk energy content as well as somatic cell score) was carried out in the German Angeln dairy cattle population. Additionally, the consideration of the physiological background of the milk production trait related QTL was part of the present study. Following a daughter design, five paternal half-sib families with a total of 805 daughters were genotyped for 43 markers on five chromosomes (chromosomes 6, 14, 16, 18, and 27). Weighted regression models were applied for the statistical analysis. Chromosomewise significant QTL were identified on chromosomes 6 and 18. QTL were segregating on chromosome 6 for the traits lactose content and protein content. On chromosome 18, QTL were found for the traits lactose yield and content, protein yield, and milk energy yield. No QTL were segregating for the other traits and on the other chromosomes, respectively.

1. INTRODUCTION

Mapping quantitative trait loci (QTL) for milk production traits in dairy cattle populations was conducted in the past years with the assistance of either the granddaughter or the daughter design (Weller et al., 1990). The characteristic of the two half-sib designs is that the DNA samples are taken from an existing population without extra mating. The granddaughter design is the favourable design in larger dairy cattle populations, because in general it is more powerful and the DNA sample collection is easier than for the daughter design. However, this design needs large male paternal half-sib groups consisting of progeny tested bulls, which are often not available in small populations. For these populations, the daughter design might be more appropriate, which uses genetic and phenotypic information of large female paternal half-sib families.

The main focus during QTL mapping in dairy cattle populations was on the six milk traits milk yield, fat yield, fat content, protein yield, protein content, and somatic cell score. For these traits QTL have been found in different breeds, e.g., in the Holstein Friesian population (Boichard et al., 2003), the Norwegian Dairy Cattle breed (Olsen et al., 2002), and the Finnish Ayrshire (Viitala et al., 2003). The yield traits, especially milk yield, are of complex nature and the QTL found for milk yield is difficult to explain physiologically. Mapping QTL for milk related traits might help to better characterise the QTL for complex traits and is a prerequisite for identifying the causal mutation underlying a complex trait QTL. Two examples, described in the literature, are the detection of the mutation in the porcine *PRKAG3* gene (protein kinase, AMP-activated, γ_3 -subunit) on pig chromosome 15 (Milan et al., 2000) and the mutation in the bovine *DGATI* (acyl-CoA:diacylglycerol acyltransferase1) gene on bovine chromosome 14 (Grisart et al., 2002; Winter et al., 2002). Milan et al. (2000) reported a nonconservative substitution (*R200Q*) in the *PRKAG3* gene. This mutation causes a low pH₂₄-value of meat from RN⁻ pigs, a reduced water-holding capacity, and therefore a reduced yield of cured cooked ham (Rendement Napole). Grisart et al. (2002) and Winter et al. (2002) identified the *DGATI* gene as a candidate gene for a QTL for milk production traits on bovine chromosome 14. A nonconservative lysine to alanine substitution (*K232A*) at position 232 affects the milk production traits, especially milk fat yield and content (Grisart et al., 2002; Winter et al., 2002). Recently, Kühn et al. (2004) investigated five VNTR (variable number of tandem repeats) alleles in the *DGATI* promoter, which showed additional effects on milk production traits. In the German Angeln population six VNTR alleles were observed. One of these alleles showed significant effects on some milk production traits compared to the other ones (see chapter two of this thesis).

The objective of this study was to map QTL for the milk production traits milk yield, protein yield and content, fat yield and content, lactose yield and content, milk energy yield and content as well as somatic cell score in the German Angeln dairy cattle population using a daughter design. The Angeln breed is located in the North of Germany and was crossed with different red breeds like Red Holstein, Swedish Red and White, and Finnish Ayrshire (Savaş et al., 1998). The traits lactose yield and content, and milk energy yield and content were included because the physiological point of view on these trait related QTL shows a possibility to explain the traditional milk production trait QTL in a better way.

2. MATERIAL AND METHODS

2.1 Pedigree data

Five paternal half-sib families of the German Angeln dairy cattle population were analysed with the assistance of a daughter design (Weller et al., 1990). These families were chosen because of their relatively large number of female half-sibs. The total number of daughters was 805 and the family size varied between 123 and 199 daughters per sire, with an average of 161 daughters.

2.2 Genotyping process

Blood samples from the daughters were collected on different farms and semen samples were taken from the five sires. No samples were taken from the daughters' dams. DNA was extracted with the silica-gel method described in a previous study (see chapter one of this thesis). Five chromosomes of special interest (BTA6, 14, 16, 18, and 27) were selected. A total of 43 markers, including the *DGATI K232A* mutation, distributed over the five chromosomes were selected from previously published bovine marker maps (USDA cattle genome marker map, 2004; INRA BOVMAP database, 2004). PCR for the markers was done on a MJ Research PTC-200 thermocycler (Global Medical Instrumentation Inc., Ramsey, Minnesota). Electrophoresis was carried out using the MegaBACE 500 Analysis System (Amersham Biosciences Europe GmbH, Freiburg, Germany). The genotyping data were stored in the ADRDB database (Reinsch, 1999). A marker-assisted estimation of pedigree errors revealed 7% conflicting daughters (i.e., genotypes of daughters and of respective sires showed a violation of the Mendelian laws) (see chapter one of this thesis). These daughters were excluded from the analysis and therefore, the total number of daughters included in the present study was 749. Multipoint marker maps were constructed using the options twopoint, build, and *flipsn* of the software CRI-MAP 2.4 (Green et al., 1990).

2.3 Phenotypic data

The following milk traits were included in this study: milk yield (MY), fat yield (FY) and content (FC), protein yield (PY) and content (PC), lactose yield (LY) and content (LC), milk energy yield (EY) and content (EC) as well as somatic cell score (SCS). Yield deviations (YD) were used as the phenotypic units of measurement. For the traits MY, FY, PY, and SCS, YD were taken from the August 2004 routine national breeding evaluation for the Angeln breed (VIT, Verden, Germany). No YD were estimated in the routine national breeding evaluation for FC and PC, EY and EC as well as LY and LC. Therefore, YD for FC and PC were calculated by using the following formula (in accordance with VIT, 2003), illustrated for PC:

$$YD(PC) = \frac{YD(PY) \times 100 - YD(MY) \times PM(PC)}{YD(MY) + PM(MY)},$$

with PM the population mean (see chapter two of this thesis). For the traits LY and LC as well as EY and EC a genetic evaluation was set up in order to obtain YD for these traits (Reinsch and Bennewitz, in preparation). The YD for the first three lactations were calculated as a weighted average (see chapter two of this thesis).

2.4 Statistical analysis

Three different weighted regression models were used for the statistical analysis. The corresponding weights were the reciprocal of the variance of the corresponding YD (see chapter two of this thesis). The first model estimated the gene substitution effect of the lysine variant at the *DGATI K232A* mutation. The phenotypes were regressed on the number of copies of the lysine variant at the *DGATI K232A* mutation, corrected for the fixed effects of the sires:

$$y_{ij} = s_i + b_1 \times x_{ij} + e_{ij}, \quad (1)$$

with y_{ij} the phenotype of the j th daughter of the i th sire, s_i the fixed effect of the i th sire, b_1 the regression coefficient, x_{ij} the number of copies (0, 1, 2) of the lysine variant, and e_{ij} the random residual.

For traits showing no significant *DGATI* effect, a multimarker regression model (Knott et al., 1996) was applied for the chromosome scans. For each cM on the chromosome the phenotypes were regressed on the corresponding QTL transition probabilities:

$$y_{ij} = s_i + b_{ik} \times tp_{ijk} + e_{ijk}, \quad (2)$$

where b_{ik} is the regression coefficient for the i th sire at the k th chromosomal position and tp_{ijk} presents the transition probability of the j th daughter from the i th sire at the k th chromosomal

position. The transition probabilities were calculated for each chromosomal position and each progeny with the software BIGMAP (Reinsch, 1999).

For traits, showing significance for the *DGATI K232A* mutation, the phenotypes were regressed simultaneously on the number of copies of the lysine variant at the *DGATI K232A* mutation and on the QTL transition probability:

$$y_{ij} = s_i + b_1 \times x_{ij} + b_{ik} \times tp_{ijk} + e_{ijk} . \quad (3)$$

The regressions of model (2) and (3) were calculated using the software ADRQLT (Reinsch, 1999). An F-test was used to find out the position of a putative QTL on the respective chromosome. The null hypothesis represents the absence of a QTL, whereas the alternative hypothesis represents the segregation of a QTL on the particular chromosome. The chromosomewise error probabilities (P_c) were calculated by the permutation test performing 10,000 permutations (Churchill and Doerge, 1994).

In total, 60 tests were conducted, that were composed of the number of tests of the three different regression models. Model (1) was evaluated for all ten traits, i.e., ten tests were conducted. The second regression model was applied for the three traits, that were not significant at the *DGATI K232A* mutation (EY, LC, and SCS) resulting in 15 tests, i.e., three traits times five chromosomes. The third model was evaluated for the other seven traits, resulting in 35 tests. The problem of multiple testing was considered by using the concept of the false discovery rate (FDR) (Benjamini and Hochberg, 1995). The FDR is loosely defined as the expected proportion of true null hypotheses within the class of rejected null hypotheses. The FDR q -value for a particular test is defined as the expected proportion of false positive, when all tests with an error probability below the error probability of this particular test are declared as significant. All 60 tests were ordered according to their error probabilities, and for each of the ordered tests a FDR q -value was estimated by taking into account the mixture distribution of the error probability using the software QVALUE (Storey and Tibshirani, 2003). It was shown that accounting for the proportion of true null hypotheses (π_0) the q -values are less conservative than assuming that every null hypothesis is true, i.e., $\pi_0 = 1$. Because of the relatively low number of performed tests, compared to, e.g., microarray experiments, the bootstrap option for the π_0 estimation of the FDR q -value was applied (Storey and Tibshirani, 2003).

The 95% confidence interval (CI95) for the QTL locations was estimated by permutation bootstrapping (Bennewitz et al., 2002), performing 250 bootstrap samples. The over-estimation of QTL effect estimates from genome scans due to biased sampling is a well

known problem. Therefore, the QTL effects in this study were estimated by a bootstrap approach as proposed by Bennewitz et al. (2003). The authors showed that this method produced QTL effect estimates with a reduced bias. In brief, 250 bootstrap samples were generated and each bootstrap sample was divided into two sets. The first set was built by the animals that were sampled during the bootstrapping, this set was termed estimation set. The second set, called test set, was built by those animals which are not sampled. The estimation set was analysed and the position on the chromosome with the highest test statistic was stored. Next, the test set was analysed, but only at the particular estimated QTL position from the estimation set, and the QTL effects, i.e., regression coefficients, were stored. Finally, the QTL effect estimate was the mean of all 250 test set estimates. This procedure was applied only for the QTL heterozygous families, i.e., the families which showed a significant QTL effect in the initial QTL scan (t-test, $P < 0.05$). The standardised substitution effects of the putative QTL were obtained by dividing the substitution effect by the additive genetic standard deviation of the respective trait.

3. RESULTS

3.1 Marker Maps

Table 1 presents all included markers for each chromosome and the results of the multipoint analysis. The number of markers per chromosome ranged between seven for BTA16 and BTA27 to eleven for BTA6. The average number of markers per chromosome was nine. On BTA18 only the region between marker BM7109 and TGLA227 was analysed.

Table 1: Chromosomes (BTA) and respective markers used in this study and included in the genetic maps

BTA	Marker and distance from the beginning of the chromosome in cM (Kosambi) (except for BTA18, which was only genotyped in the region from BM7109 to TGLA227)
06	INRA133 0.0, BM1329 28.1, BM143 45.0, TGLA37 47.3, DIK082 49.4, BM4528 62.9, BM415 101.0, <i>CSN3</i> 121.7, <i>CSN1S1</i> 121.7, BP7 135.4, BMC4203 152.2
14	<i>DGATI</i> 0.0, ILSTS039 3.9, CSSM066 9.9, BMS1678 18.7, ILSTS011 20.2, RM180 28.2, BM302 49.1, BMS1899 61.1, BM4513 88.3, BM6425 114.4
16	MGTG1 0.0, HUI614 10.3, BM1311 40.8, CSSM028 70.0, INRA048 88.5, HUI625 105.3, BMS719 106.3
18	BM7109 0.0, ILSTS002 16.3, BMS2639 21.2, BMS833 34.2, BMS2785 40.2, BM2078 50.1, BM6507 55.9, TGLA227 66.7
27	BM3507 0.0, TGLA179 6.0, RM209 15.4, CSSM043 34.8, INRA134 42.5, INRA027 47.3, BM203 56.7

3.2 QTL RESULTS

Significant positive average allele substitution effects of the lysine variant at *DGATI K232A* were observed for the content traits PC, FC, and EC as well as for FY. Significant negative effects were observed for the yield traits MY, PY, and LY (results of model (1); for a detailed description of the *DGATI K232A* effects in the Angeln population see chapter two of this thesis). The results of the second model identified chromosomewise significant QTL ($P_c \leq 0.05$) on BTA6 and BTA18. On BTA6, a QTL for LC was segregating close to the casein complex and the marker BP7 at position 132 cM. On BTA18, a QTL for the same trait is segregating at position 17 cM near by ILSTS002. Another QTL was segregating on BTA18 for EY around marker BM2078.

Model (3) identified chromosomewise significant QTL for the traits PC (BTA6), LY (BTA18), and PY (BTA18). On BTA6, a putative QTL for PC was localised at position 152 cM (BMC4203). On BTA18, a QTL for LY was localised at position 50 cM around BM2078. A QTL for PY was located near the marker BM2078 at position 50 cM. No significant segregation of a QTL was detected for the other traits and on the other chromosomes, respectively. Figure 1 presents the plots for the test statistics for the two traits LC and PC on BTA6. Figure 2 presents the different plots of the test statistics for the four traits LC, EY, LY,

and PY on BTA18. All displayed plots of the test statistics did not show very pronounced peaks for the respective QTL positions.

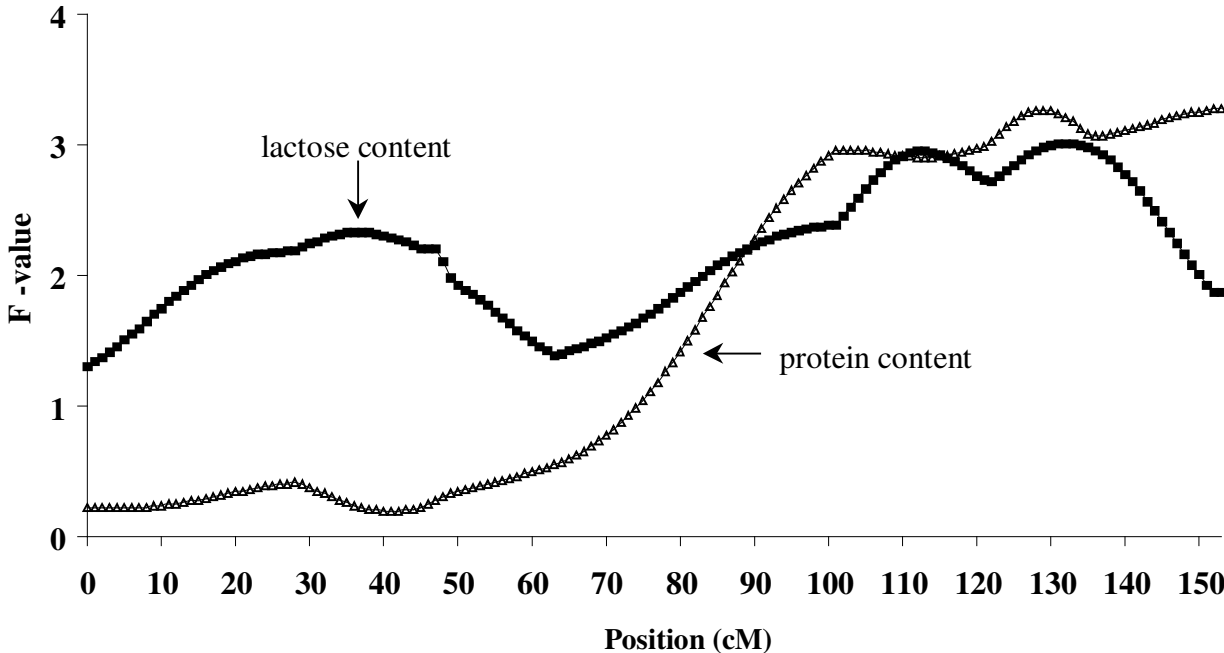


Figure 1: Test statistics for the traits lactose content ($P_c = 0.06$) and protein content ($P_c = 0.03$) on chromosome 6.

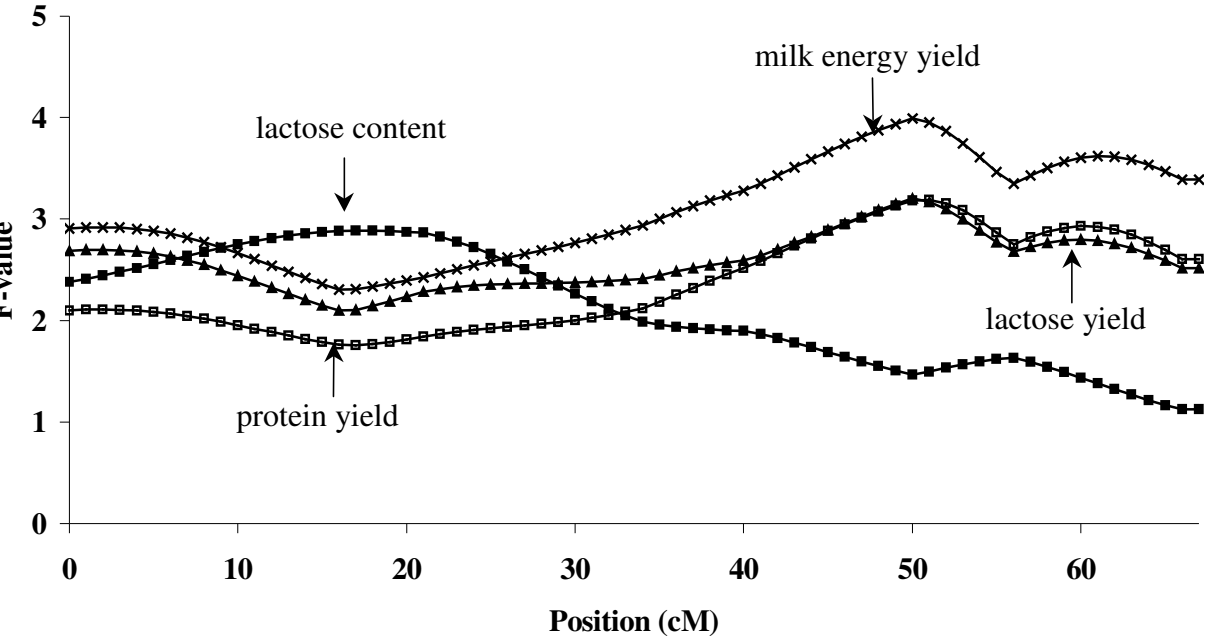


Figure 2: Test statistics for the traits lactose content ($P_c = 0.05$), milk energy yield ($P_c = 0.007$), lactose yield ($P_c = 0.03$), and protein yield ($P_c = 0.03$) on chromosome 18.

The estimated FDR q -values are presented in Table 2 for the 16 most significant hypotheses. In addition to the q -values, the chromosomewise and comparisonwise error probabilities are listed, respectively. The proportion of truly null hypotheses was $\hat{\pi}_0 = 0.771$. Therefore, the number of expected significant tests was $(1 - 0.771) \times 60 \approx 14$.

Table 2: Results of the 16 most significant tests with F-value, chromosomewise (P_c) and comparisonwise (P_{comp})¹ error probabilities (model (1) to (3)), respectively, and the FDR q -values for the different traits on different chromosomes (BTA)

Rank	Trait ²	BTA	Model	F-value	P_c or P_{comp}	q -value
1	LY	14	1	20.59	<0.0001 ¹	0.0015
2	FC	14	1	177.64	<0.0001 ¹	0.0015
3	EC	14	1	248.89	<0.0001 ¹	0.0015
4	PC	14	1	59.23	<0.0001 ¹	0.0015
5	MY	14	1	30.68	<0.0001 ¹	0.0015
6	FY	14	1	14.51	0.0002 ¹	0.0015
7	EY	18	2	3.99	0.0070	0.0463
8	PY	14	1	5.78	0.0164 ¹	0.0948
9	PC	06	3	3.28	0.0315	0.1362
10	PY	18	3	3.19	0.0318	0.1362
11	LY	18	3	3.21	0.0324	0.1362
12	LC	18	2	2.89	0.0537	0.2070
13	LC	06	2	3.01	0.0600	0.2135
14	PY	06	3	2.86	0.0860	0.2778
15	LC	14	1	2.88	0.0901 ¹	0.2778
16	MY	14	3	2.78	0.1083	0.3131

²The abbreviations are as follows: EY = milk energy yield, EC = milk energy content, FY = fat yield, FC = fat content, LY = lactose yield, LC = lactose content, MY = milk yield, PY = protein yield, PC = protein content, (SCS = somatic cell score).

Table 3 represents the results of the estimation of the 95% confidence intervals and of the QTL effect estimate. The CI95 for the traits on BTA18 covered mostly the whole investigated chromosomal region. The CI95 for LC on BTA6 ranged from 21 cM to 150 cM, whereas the CI95 for PC ranged from 88 cM to 152 cM. In general, the 95% CI for the QTL locations were rather wide. The standardised substitution effects were obtained by dividing the substitution effect by the additive genetic standard deviation of the respective trait and are

presented for the traits PY, EY, LC, and LY. The standardised substitution effects for the putative QTL ranged from 0.10 to 0.29.

Table 3: Results of the QTL analysis with 95% confidence interval (CI95), substitution effects (α), and estimated position for the putative QTL on the different chromosomes (BTA) for the QTL heterozygous families

BTA	No. of heterozygous families	Trait ¹	CI95 (cM)	Position (cM)	α
06	1	PC	88–152	152	– ²
06	2	LC	21–150	132	0.17
18	2	PY	3–65	51	0.15
18	3	EY	1–65	50	0.12
18	3	LY	1–65	50	0.10
18	1	LC	6–61	17	0.29

¹ See Table 2 for abbreviations.

² No additive genetic standard deviation available.

4. DISCUSSION

4.1 Physiological background of milk production QTL

It is well known, that the amount of lactose controls the milk yield during the lactation because of the osmotic effect of the lactose in the alveolar cells in the final step of the lactose synthesis. Consequently, the mapping of QTL for lactose yield and lactose content is a suitable basis for the mapping of genes influencing the milk yield production complex. Until now, only a few studies observed the genetic background of the trait lactose. Lundén et al. (1997) and Tsiaras et al. (2005) investigated the relationship between genetic variants for milk protein and, among other traits, lactose. Lundén et al. (1997) revealed that the A_1A_1 genotype of the β -casein locus, which is located on chromosome 6, was related to lower lactose yield for the Swedish Red and White breed. Tsiaras et al. (2005) presented an association between a higher production of lactose and the AB genotype of the β -lactoglobulin locus in Holstein Friesian, that can be affected by the influence of the AB genotype on milk yield. No significant associations between the κ -casein locus and the traits lactose yield and content were reported in the study of Tsiaras et al. (2005). In contrast, Strzalkowska et al. (2002) investigated higher lactose content values for the AA genotype at the κ -casein locus (*CSN3*) in the Polish Black-and-White cattle. In our study, a QTL was segregating near the casein

complex for lactose yield on BTA6. This QTL supported the prementioned relationship between the κ -casein locus and lactose.

Milk energy is another parameter to describe the metabolic activity of the milk production process. Nostitz and Mielke (1995) estimated a highly significant positive correlation between milk energy content and the two traits protein and fat content ($r > 0.9$). On the other hand, a QTL on BTA18 was significant for milk energy yield but not for fat yield, indicating that despite of the high genetic correlation on a polygenic level, not all QTL affect significantly both traits, fat yield and milk energy yield.

4.2 Segregating QTL for production traits

A putative QTL was segregating for protein content on chromosome 6. On the same chromosome, Olsen et al. (2002) reported a QTL with alleles causing a reduction in protein and fat content and an increase in milk yield next to marker BM143 in the Norwegian Dairy Cattle population. Recently, Cohen-Zinder (2005) identified a polymorphism encoding a substitution of tyrosine-581 to serine (*Y581S*) in the *ABCG2* transporter, which was associated with an increase of protein and fat content and a decrease of milk yield, next to BM143. The authors suggested that *ABCG2(2)* is the causative mutation for the QTL affecting these traits. Another QTL on this chromosome was found in our study for lactose yield next to the casein complex, where Boichard et al. (2003) located a QTL for protein content (near by INRAK) in the French Holstein, Normande, and Montbéliarde dairy cattle breeds. On BTA18, QTL segregating for the traits milk energy, lactose, and protein yield were presented in this study at the same position (50 cM). Hence, it could be a single pleiotropic QTL that affects the three traits. In the same region Olsen et al. (2002) reported a significant QTL for protein yield. Additionally, Ashwell et al. (2004) found a segregating QTL for fat yield in the same region in a Holstein Friesian population. Furthermore, a putative QTL was found for milk yield ($P_c = 0.15$) and another one for lactose yield on BTA18, which is in accordance with Olsen et al. (2002) who reported a significant QTL segregating for milk yield on the same chromosome. Given this QTL is real, the highly significant error probability of the QTL affecting milk yield might be the result of the low power of our study. These results support the suggestion that the main part of the QTL segregating for milk yield are depending on genetic factors integrated in the lactose synthesis and secretion (Viitala et al., 2003).

The confidence intervals for the QTL positions were wide on both chromosomes. This corresponds well with the relatively flat curves of the test statistic plots (Figure 1 and 2). One

reason was that the population size as well as the effects of the different QTL were small, resulting in a low experimental power of the study.

4.3 FDR analysis

In common mapping studies the significance threshold levels, to control the Type-I error, will be defined with the aid of the permutation test. One problem of these significance criteria is, that they are trait-specific and in the case of stringent thresholds the Type-II error (missing of present QTL) will increase. Hayes and Goddard (2001) pointed out that especially QTL with smaller effects will be missed with the former methods. The FDR analysis, as proposed by Storey and Tibshirani (2003), was conducted to avoid this. In the recent years, different methods were evaluated for FDR estimation purposes, which differ from the practical point of view mainly in the estimation of the proportion of true null hypotheses, π_0 . Benjamini and Hochberg (1995) set $\pi_0 = 1$, because they assumed that π_0 is unknown. In contrast to Benjamini and Hochberg (1995), Storey and Tibshirani (2003) estimated π_0 from a mixture distribution of the error probabilities. By taking this mixture distribution into account, the FDR provides a natural balance between the true positive and the false positive QTL compared to the error probabilities. The results of the FDR analysis can be taken as a suggestion list for further studies, e.g., QTL confirmation.

4.4 Marker-assisted selection in small dairy cattle populations

The genotypic information of the sires and their daughters could be used for marker-assisted selection (MAS). For small populations, like the Angeln population, the bottom-up approach is a possible method for the preselection of young bulls before entering the progeny testing (Mackinnon and Georges, 1998). Daughters of a sire are genotyped and divided into two groups depending on the haplotype they receive from their sire (in accordance with the daughter design). The different values of the average yield deviations for the two daughter groups give information which sire is heterozygous for the respective QTL. In the final step, only the sons of the heterozygous sires are selected for the progeny testing, that received the favourable haplotype of their sire. The preselection of young bulls can help to optimise the time consuming and costly progeny testing programmes. The benefits of this kind of MAS can be seen in three different ways. Firstly, in the case of a reduction of the total number of test bulls per year and a constant test capacity, the insemination per test bulls can be increased. Hence, the accuracy of the estimated breeding values for these bulls will be increased, especially for traits with a low heritability. A second possibility is a reduction of

the test capacity, as a result of a reduced number of test bulls and a constant number of inseminations. This will result in a higher financial benefit of the breeding organisations. Thirdly, in the case of a constant test capacity, and therefore, a constant number of test bulls, the selection intensity can be increased by testing a higher number of bulls. In small breeds, like the Angeln breed, MAS could be an alternative to overcome the disadvantage of the small population size, and hence, to make the breed more competitive. However, a main challenge during the application of bottom-up MAS is the organisation of the blood or tissue collection of the daughters for the DNA extraction. This is necessary in order to test whether sires are heterozygous for a QTL and derive the family-specific marker-QTL linkage phases. Hence, especially for small populations it would be beneficial to have markers that show a linkage disequilibrium with the QTL, because in these cases the corresponding linkage phases are more or less constant across families (depending on the strength of the linkage disequilibrium) and have not to be identified for each family separately by bottom-up. Obviously, the most promising situation for a DNA-assisted selection is to have knowledge about the causative mutation. For the Angeln population known causative mutations affecting milk production traits and also udder health are the DGAT1 K232A mutation and a mutation in the DGAT1 promoter region (see chapter two of this thesis). The two mutations can directly be used for a gene-assisted selection in the Angeln population.

5. CONCLUSIONS

The objective of this study was to map QTL for ten milk production traits on five chromosomes. QTL for protein, lactose, and milk energy yield as well as for protein and lactose content were reported. These results are one step in the understanding of the physiological nature of the complex milk production QTL and could be the basis for further investigations.

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REFERENCES

- Ashwell, M. S., D. W. Heyen, T. S. Sonstegard, C. P. Van Tassell, Y. Da, P. M. VanRaden, M. Ron, J. I. Weller, and H. A. Lewin. 2004. Detection of quantitative trait loci affecting milk production, health, and reproductive traits in Holstein cattle. *J. Dairy Sci.* 87(2):468–475.
- Benjamini, Y., and Y. Hochberg. 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J. R. Stat. Soc. Ser. B* 57:289–300.
- Bennewitz, J., N. Reinsch, and E. Kalm. 2002. Improved confidence intervals in quantitative trait loci mapping by permutation bootstrapping. *Genetics* 160(4):1673–1686.
- Bennewitz, J., N. Reinsch, and E. Kalm. 2003. Comparison of several bootstrap methods for bias reduction of QTL effect estimates. *J. Anim. Breed. Genet.* 120(6):403–416.
- Boichard, D., C. Grohs, F. Bourgeois, F. Cerqueira, R. Faugeras, A. Neau, R. Rupp, Y. Amigues, M. Y. Boscher, and H. Levéziel. 2003. Detection of genes influencing economic traits in three French dairy cattle breeds. *Genet. Sel. Evol.* 35(1):77–101.
- Churchill, G. A., and R. W. Doerge. 1994. Empirical threshold values for quantitative trait mapping. *Genetics* 138(3):963–971.
- Cohen-Zinder, M., E. Seroussi, D. M. Larkin, J. J. Loo, A. Everts-van der Wind, J.-H. Lee, J. K. Drackley, M. R. Band, A. G. Hernandez, M. Shani, H. A. Lewin, J. I. Weller, and M. Ron. 2005. Identification of a missense mutation in the bovine *ABCG2* gene with major effect on the QTL on chromosome 6 affecting milk yield and composition in Holstein cattle. *Genome Res.* 15(7):936–944.
- Green, P., K. Falls, and S. Crooks. 1990. Documentation for CRI-MAP, Version 2.4., Washington University School of Medicine, St. Louis, MO.
- Grisart, B., W. Coppieters, F. Farnir, L. Karim, C. Ford, P. Berzi, N. Cambisano, M. Mni, S. Reid, P. Simon, R. Spelman, M. Georges, and R. Snell. 2002. Positional candidate cloning of a QTL in dairy cattle: identification of a missense mutation in the bovine *DGAT1* gene with major effect on milk yield and composition. *Genome Res.* 12(2):222–231.
- Hayes, B., and M. E. Goddard. 2001. The distribution of the effects of genes affecting quantitative traits in livestock. *Genet. Sel. Evol.* 33(3):209–229.
- INRA BOVMAP database. 2004. <http://locus.jouy.inra.fr/>.
- Knott, S. A., J. M. Elsen, and C. S. Haley. 1996. Methods for multiple-marker mapping of quantitative trait loci in half-sib populations. *Theor. Appl. Genet.* 93(1-2):71–80.
- Kühn, Ch., G. Thaller, A. Winter, O. R. P. Bininda-Emonds, B. Kaupe, G. Erhardt, J. Bennewitz, M. Schwerin, and R. Fries. 2004. Evidence for multiple alleles at the *DGAT1*

- locus better explains a quantitative trait locus with major effect on milk fat content in cattle. *Genetics* 167(4):1873–1881.
- Lundén, A., M. Nilsson, and L. Janson. 1997. Marked effect of β -lactoglobulin polymorphism on the ratio of casein to total protein in milk. *J. Dairy Sci.* 80(11):2996–3005.
- Mackinnon, M. J., and M. A. J. Georges. 1998. Marker-assisted preselection of young dairy sires prior to progeny-testing. *Livest. Prod. Sci.* 54(3):229–250.
- Milan, D., J.-T. Jeon, C. Looft, V. Amarger, A. Robic, M. Thelander, C. Rogel-Gaillard, S. Paul, N. Iannuccelli, L. Rask, H. Ronne, K. Lundström, N. Reinsch, J. Gellin, E. Kalm, P. Le Roy, P. Chardon, and L. Andersson. 2000. A mutation in *PRKAG3* associated with excess glycogen content in pig skeletal muscle. *Science* 288(5469):1248–1251.
- Nostitz, B., and H. Mielke. 1995. Vergleich verschiedener Methoden der Bestimmung des Milchenergiegehaltes beim Schwarzbunten Milchrind. *J. Anim. Physiol. Anim. Nutr.* 73(1):9–18.
- Olsen, H. G., L. Gomez-Raya, D. I. Våge, I. Olsaker, H. Klungland, M. Svendsen, T. Ådnøy, A. Sabry, G. Klementsdaal, N. Schulman, W. Krämer, G. Thaller, K. Rønningen, and S. Lien. 2002. A genome scan for quantitative trait loci affecting milk production in Norwegian Dairy Cattle. *J. Dairy Sci.* 85(11):3124–3130.
- Reinsch, N. 1999. A multiple-species, multiple-project database for genotypes at codominant loci. *J. Anim. Breed. Genet.* 116(8):425–435.
- Savaş, T., N. Reinsch, and E. Kalm. 1998. Auswirkungen der Rassenzusammensetzung auf Merkmale der Tagesmilchmenge und auf die Zellzahl beim Angler Rind. *Arch. Tierz.* 41(3):201–209.
- Storey, J. D., and R. Tibshirani. 2003. Statistical significance for genomewide studies. *Proc. Natl. Acad. Sci. USA* 100(16):9440–9445.
- Strzalkowska, N., J. Krzyżewski, L. Zwierzchowski, and Z. Ryniewicz. 2002. Effects of κ -casein and β -lactoglobulin loci polymorphism, cows' age, stage of lactation and somatic cell count on daily milk yield and milk composition in Polish Black-and-White cattle. *Anim. Sci. Papers and Reports* 20(1):21–35.
- Tsiaras, A. M., G. G. Bargouli, G. Banos, and C. M. Boscos. 2005. Effect of kappa-casein and beta-lactoglobulin loci on milk production traits and reproductive performance of Holstein cows. *J. Dairy Sci.* 88(1):327–334.
- USDA cattle genome marker maps. 2004. <http://www.marc.usda.gov/genome/genome.html>.

- Viitala, S. M., N. F. Schulman, D. J. de Koning, K. Elo, R. Kinos, A. Virta, J. Virta, A. Mäki-Tanila, and J. H. Vilkki. 2003. Quantitative trait loci affecting milk production traits in Finnish Ayrshire dairy cattle. *J. Dairy Sci.* 86(5):1828–1836.
- VIT. 2003. Zuchtwertschätzung, Vereinigte Informationssysteme Tierhaltung w.V., Verden/Aller, Germany. <http://www.vit.de>.
- Weller, J. I., Y. Kashi, and M. Soller. 1990. Power of daughter and granddaughter designs for determining linkage between marker loci and quantitative trait loci in dairy cattle. *J. Dairy Sci.* 73(9):2525–2537.
- Winter, A., W. Krämer, F. A. O. Werner, S. Kollers, S. Kata, G. Durstewitz, J. Buitkamp, J. E. Womack, G. Thaller, and R. Fries. 2002. Association of a lysine-232/alanine polymorphism in a bovine gene encoding acyl-CoA:diacylglycerol acyltransferase (*DGATI*) with variation at a quantitative trait locus for milk fat content. *Proc. Natl. Acad. Sci. USA* 99(14):9300–9305.

CHAPTER FOUR

General Discussion

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GENERAL DISCUSSION

The overall aim of this thesis was the marker-assisted estimation of pedigree errors and the mapping of quantitative trait loci (QTL) in the Angeln dairy cattle population. The use of microsatellite markers for paternity verification is the main objective in **chapter one**. Furthermore, the effects of pedigree errors (wrong and missing sire information) on the genetic gain in the German Angeln population were estimated. In **chapter two** the characterisation of the evidence and of the frequencies of the alleles at the *DGATI K232A* mutation, the *DGATI* promoter VNTR, and the *CSN1S1* promoter on the bovine chromosome 14 (BTA14) and BTA6 is the main objective. Another aspect of this chapter is the investigation of the effects of their alleles on the milk production traits. Finally, the mapping process of QTL affecting the milk production traits is presented in **chapter three**. One objective is the characterisation of milk trait related QTL, i.e. putative QTL for the traits lactose yield and content as well as milk energy yield and content. In the present chapter additional aspects of the prementioned results are discussed.

Effects of pedigree errors on different breeding aspects in dairy cattle populations

The use of microsatellite markers for the estimation of wrong sire information (WSI) in the German Angeln population is discussed in **chapter one**. Additionally, the impact of wrong and missing sire information (MSI) on the reliability of estimated breeding values and on the genetic gain is investigated.

Christensen et al. (1982) and Weller et al. (2004) mentioned seven different sources for paternity errors which can be show up on the dairy farm, the AI-centre, or in the genotyping laboratory. Christensen et al. (1982) mentioned different reasons for paternity errors: 1) the AI-centre or the technician could mistake the semen of one bull for the semen of another; 2) further insemination of a pregnant cow; 3) the last insemination is sometimes incorrectly registered due to a misprinting of the bull's herdbook number or name; 4) the use of natural service bulls may lead to pregnancy of cows which have beforehand been served by AI-bulls and are supposed to be pregnant; 5) interchange of calves is likely to be more and more frequent due to increasing herd size; 6) trade in calves and heifers may give rise to incorrect pedigree information. As a seventh factor, Weller et al. (2004) included mistakes in the paternity made by a laboratory, which result in rejection of paternity of cows with correct paternity identification. In recent years, microsatellite markers became the international standard system of paternity verification in dairy cattle breeds (Bredbacka and Koskinen, 1999). The advantage of using microsatellites for pedigree verification, compared to blood

groups and protein polymorphisms, is their polymorphic character (Ron et al., 1996). The probability of detecting wrong paternity will increase with highly polymorphic markers. Ron et al. (1996) determined that in former times the true misidentification rate was underestimated because of lowly polymorphic markers. Another criterion of the identification of wrong paternity is the number of putative sire alleles in the population. Consequently, the probability of exclusion will increase when the alleles of the putative sire are rare in the population (Ron et al., 1996).

In the Angeln population the estimated WSI was 7% and the MSI was around 10%. The result of the WSI is in accordance with the estimated proportions between 3 and 23% in the Holstein Friesian breed (e.g., Weller et al., 2004). Further investigations in this study showed that WSI and MSI combined their impact on the genetic gain. Additionally, the calculations put out that the impact of WSI was more harmful than the impact of MSI. Van Vleck et al. (1970a, b) and Christensen et al. (1982) pointed out that the number of daughters per sire can influence the impact of WSI. In more detail, the effect of WSI will be more serious in small progeny groups than in larger ones. The increase of the number of progenies is, especially in smaller populations like the Angeln breed, a capacious problem because of the limited test capacity in the progeny testing period. This results in a more harmful impact of pedigree errors in small populations than in larger populations. In the present study it was shown that the genetic gain for the Angeln population will decrease with a higher impact of WSI and MSI. Geldermann et al. (1986) estimated a decrease of genetic gain between 8.7% ($h^2 = 0.5$) and 16.9% ($h^2 = 0.2$) at a WSI of 15% compared with a situation without WSI.

Another type of pedigree error is the wrong or missing dam information, but until now not much is known about this kind of error probability. The proportion of cows with unknown dam and known sire in the Angeln population is around 0.09% (Reinhardt, F.; Germany, personal communication), whereas the proportion of cows with unknown dam and unknown sire is around 1.72%.

Chapter one emphasises that pedigree errors reduce the genetic progress. To avoid pedigree errors, the breeding organisations have to control their verification system. Another possibility will be to increase the number of daughters per sire, which could be a problem in small dairy cattle populations. To ensure the prevention of WSI, bull dams and sires have to be genotyped before entering the testing process.

The *DGATI* K232A mutation on bovine chromosome 14: an overview

The investigation of QTL affecting milk production traits in different dairy cattle populations has been the main objective of mapping studies in the last decade. These traits are mainly of a complex and heterogeneous nature, e.g., milk yield, making it difficult to detect the responsible genes for the marked QTL. Hence, it is getting increasingly important to identify the genetic background of these special traits. One example in literature is the *DGATI* gene (acyl-CoA:diacylglycerol acyltransferase1 gene). Cases et al. (1998) reported that the *DGATI* gene encodes an enzyme catalysing the final step of the triglyceride synthesis using diacylglycerol and fatty acyl-CoA as substrates. Smith et al. (2000) investigated that *DGATI*-deficient mice are unable to lactate but they are viable and fertile. The authors concluded that *DGATI* is substantial for the lactation and the development of the mammary gland.

Several mapping studies investigated a QTL at the centromeric end on the bovine chromosome 14 (BTA14) affecting the milk production traits, and especially milk fat content, with the assistance of an identity-by-descent method or by linkage disequilibrium (e.g., Riquet et al., 1999; Farnir et al., 2002). Grisart et al. (2002) and Winter et al. (2002) investigated the *DGATI* gene as a potential candidate gene affecting milk fat content. A nonconservative lysine to alanine substitution (*K232A*) at position 232 affects the milk production traits, especially milk fat yield and content (Grisart et al., 2002; Winter et al., 2002; Grisart et al., 2004). This nonconservative substitution is caused by an adenine/adenine to guanine/cytosine dinucleotide substitution at position 10433 and 10434 in exon number VIII. A higher milk fat content is caused by the lysine variant, whereas the lower milk fat content is determined by the alanine variant which could have a negative effect on the acyl-CoA-binding capacity of the *DGATI* gene (Winter et al., 2002). The authors concluded that the lysine variant presents the ancestral state of the diallelic *DGATI* *K232A* mutation. In a further study, Grisart et al. (2004) reported that the lysine variant has been under a positive selection process supporting the effects on the functionality of the *DGATI* gene, which is in accordance with the selection objectives in the different dairy cattle populations, e.g. German Angeln dairy cattle population.

In an associated expression study, it was shown that there is no difference in the mRNA expression levels for the two *DGATI* variants (Grisart et al., 2004). In a further step it was shown that the lysine variant affects V_{\max} of the enzyme in an increasing direction, resulting in an increased triglyceride synthesis, i.e. a higher fat content. The authors found that the V_{\max} of the lysine variant is superior to the V_{\max} of the alanine variant.

In **chapter two** the allele frequencies and the substitution effects of the *DGATI K232A* mutation in the German Angeln dairy cattle population were investigated. The allele frequency of the lysine variant was higher than in other German dairy cattle breeds (Thaller et al., 2003a; Bennewitz et al., 2004), which is the result of previous breeding objectives in the Angeln population. In view of the modification of the relative breeding value milk yield (VIT, Verden, 2004, Germany) in the German Angeln dairy cattle population, it might be possible that the allele frequency of the lysine variant will adapt to the frequencies in the Holstein Friesian population. More specifically, the breeding objectives in the Angeln population will change from a higher fat yield to a higher milk yield with more emphasis on protein yield. The substitution effects of the lysine variant at the *DGATI K232A* mutation were substantially lower in the Angeln breed than in other German dairy cattle breeds (Thaller et al., 2003a; Bennewitz et al., 2004). The reason could be the general selection on higher fat and protein contents in this breed, resulting in a higher level of the triglyceride synthesis compared to the Holsteins. The limiting factor for a higher triglyceride synthesis caused by the lysine variant in the Angeln population might be the limited availability of the two substrates diacylglycerol and fatty acyl-CoA.

Additional effects responsible for the milk production QTL on BTA14 beside the diallelic *DGATI K232A* mutation were found (e.g., Bennewitz et al., 2004). In Turkish women, Ludwig et al. (2002) reported five polymorphisms in the human *DGATI* promoter with additional effects on the body weight. The promoter allele *79T* showed a reduced promoter activity and is, therefore, associated with, e.g., a lower body mass index in Turkish women.

In cattle, Bennewitz et al. (2004) discussed different kinds of additional sources of genetic variation beside the diallelic *DGATI K232A* mutation. The first hypothesis is that there is another allele segregating at the *DGATI K232A* mutation, the second one is that there is an adjacent QTL which is in linkage with the *DGATI K232A* mutation, or the third possibility is that *DGATI* shows interactive effects with another locus. Recently, Kühn et al. (2004) investigated five VNTR (variable number of tandem repeats) alleles in the *DGATI* promoter, that showed additional effects on the milk production traits.

The effects of the alleles at the *DGATI* promoter VNTR in the German Angeln dairy cattle population are discussed in chapter two. In this population six different VNTR alleles were investigated, of these the sixth allele showed up only in two unrelated daughters and were excluded from the statistical analysis. The VNTR allele E showed significant effects on the milk production traits lactose yield and content, milk energy content, and somatic cell score compared to the other alleles. The VNTR allele E showed up mainly with the lysine variant at

the *DGATI K232A* mutation, whereas Kühn et al. (2004) reported that the promoter VNTR allele 5, probably the same as our VNTR allele E, showed up with the alanine variant. It has to be kept in mind that, in contrast to Kühn et al. (2004), different primer pairs for the amplification of the *DGATI* promoter VNTR were chosen. Because of the GC-rich sequences of the chosen primers by Kühn et al. (2004), we had problems with the amplifications and generated a new primer pair in the *DGATI* promoter VNTR region. The results of the present study confirmed the hypothesis of an additional source of variation due to the QTL on BTA14 affected beside the diallelic *DGATI K232A* mutation (Winter et al., 2002; Bennewitz et al., 2004; Kühn et al., 2004).

The *DGATI K232A* mutation did not solely affect milk production traits in dairy cattle populations. Several studies presented positive effects of the lysine variant for meat quality traits, i.e., fat related traits, in beef cattle. Thaller et al. (2003b) reported a significant effect of the lysine variant at the *DGATI K232A* mutation on intramuscular fat content in a German Holstein population. In contrast Moore et al. (2003) could not find an association between the *DGATI K232A* mutation and backfat in a commercial line of *Bos taurus*. The authors suggested that there might be other alleles of *DGATI* or other genes in the proximal region on BTA14 showing an effect on this trait.

The results of the present and of previously studies showed significant effects of the *DGATI K232A* mutation in dairy and beef cattle populations, but there is still a requirement to investigate the complex nature of gene regulation mechanism. Hence, it has to be known if there are additional mutations in the *DGATI K232A* gene or another polygenic gene at this chromosomal region.

Effects of the alleles at the *CSN1S1* promoter on BTA6

Different studies investigated the influence of multiple QTL on BTA6 affecting the milk production traits in dairy cattle populations, especially on protein content (e.g., Velmala et al., 1999; Ron et al., 2001; Olsen et al., 2005). A frequently reported QTL location is the casein cluster with four different linked genes (α_{s1} -casein (*CSN1S1*), β -casein (*CSN2*), α_{s2} -casein (*CSN1S2*), and κ -casein (*CSN3*)), which showed an influence on the milk production traits (Velmala et al., 1999). Jann et al. (2002) reported a new method for the differentiation of the two *CSN1S1* alleles in the coding region of *CSN1S1*. In their study, the authors investigated a nearly-fixation of the *CSN1S1* Bb haplotype in the Angeln dairy cattle population. This could be the consequence of the selection on the milk content traits in this breed. In contrast Prinzenberg et al. (2003) reported allelic variation in the proximal *CSN1S1* promoter region in

the German Holstein Friesian population. The authors investigated that allele 4 was superior compared to the other three alleles. This result could not be approved in the present study (chapter two). Maybe this is caused by the low number of daughters carrying this allele. In the German Angeln dairy cattle population two alleles (allele 2 and 3) showed superior effects mainly on the yield traits compared with the other alleles. No significant effects were found for the content traits and somatic cell score. On the other hand, Prinzenberg et al. (2003) investigated significant effects for allele 4 on protein content. Therefore, the authors suggested that allele 4 increases protein content and it could be concluded that the *CSN1S1* promoter presents a functional candidate locus for protein content. This hypothesis has to be taken into account because of the results in the Angeln population. Another option is that the effects of the *CSN1S1* promoter are caused by linkage disequilibrium with the causative gene.

The use of different experimental designs in QTL mapping studies

The first step of a QTL mapping study is the selection of an experimental design. The different types of an experimental design can be divided into two groups. One group contains the inbred line crosses, which include the following designs: backcross design, F₂-design, and recombinant inbred lines. The typical character of these designs is that they are planned designs with extra mating. Furthermore, the characterisation of QTL is much easier in these designs because the assumption is, that there are only two alleles at each locus. Therefore, inbred lines are more powerful than experimental designs of segregating populations. Besides the costs to set up these designs, another disadvantage is that the mapped QTL cannot be used directly for marker-assisted selection (MAS), because it is unknown whether they are segregating in those populations where selection takes place.

In case of dairy cattle populations, designs out of segregating populations were used as experimental design to determine the linkage between marker and QTL. The main difference to the designs discussed above is, that no extra matings have to be done. Additionally, some markers are not informative for the whole population and a putative QTL is not segregating in all families.

The main experimental designs of mapping studies in dairy cattle populations are the two half-sib designs daughter and granddaughter design (Weller et al., 1990). In the case of the granddaughter design only the grandsires and their sons (sires) were genotyped, and phenotypic data of the daughters were used. The granddaughter design is the favourable design in large dairy cattle populations, e.g., Holstein Friesian, because in general it is more powerful and the DNA sample collection is easier than for the daughter design (Weller et al.,

1990). However, this design needs large male paternal half-sib groups consisting of progeny tested bulls, which are often not available in small populations like the Angeln dairy cattle population. For small populations the daughter design might be more appropriate, and this was used in the present study. In this case, the sires and their daughters were genotyped, and additionally the phenotypic data of the daughters were used to determine the linkage between marker and QTL. The power of this design depends on the number of genotyped daughters per family and on the number of included families (Weller et al., 1990). More precisely, the power of the study increases with an increasing number of families and with an increasing number of genotyped daughters per family.

Is it possible to implement marker-assisted selection in small dairy cattle populations?

In the last decade, the main focus of the breeding objectives has changed in this manner that the functional traits getting increasingly important. Especially, for traits with a low heritability it is important to evaluate an MAS breeding scheme. In this chapter, it has to be discussed if there is a chance to implement MAS in small dairy cattle populations like the Angeln breed. The breeding situation in small dairy populations is more complicated than in larger ones because of, e.g., the small number of paternal half-sibs and the reduced test capacity. For these small populations the implementation of the bottom-up approach (Mackinnon and Georges, 1998) might be more appropriate, because of the lacks of large male paternal half-sibs but on the other hand the existence of large female paternal half-sibs. The classical bottom-up approach as proposed by Mackinnon and Georges (1998) includes basically three steps: (a) the determination of the sires' heterozygosity at the QTL, (b) the determination of the marker-QTL linkage phase (i.e., which marker haplotype is associated with the superior and inferior QTL allele, respectively), and (c) MAS of the sons, based on the probability that they have received either the positive or the negative allele of the QTL from their sires. Spelman (2002) and Stella et al. (2002) mentioned the following limitations: First of all, it rises the question about the appropriate threshold level for the determination of the QTL heterozygosity of the sire, and furthermore, the assumption is that the better (poorer) marker haplotype of the sire is superior (inferior) to the marker haplotype the son received from the dam population. The possibility of recombination between QTL and marker increases per generation. Finally, the bottom-up approach allows only MAS preselection within families, which results in the need of a generation of full-sib or similar half-sib groups of candidates. Especially, the last point has shown to be the main difficulty in setting up such a classical bottom-up approach (Spelman, 2002). These limitations can partly be overcome by the use of

MA-BLUP estimated breeding values as selection criteria for MAS. In principle, the MA-BLUP models use the same information as the classical bottom-up approach. However, because the effects of the gametes at the QTL are treated as random, they show the following properties: They account for the uncertainty about the co-inheritance of the marker haplotype and the corresponding QTL allele by the use of the gametic IBD matrix at the QTL, and they assume no artificial ranking of the superior marker haplotypes inherited from the sire and the dam population a priori. Further, a determination of the sires heterozygosity is not necessary. If the sire is homozygous at the QTL, the estimates of its two QTL gametes would be on a similar level. The estimates are unambiguously interpretable, and are comparable across families. This offers the opportunity to apply MAS for young bulls that are not full-sibs or similar half-sibs. Consequently, the pressure to increase the female reproductive capacity to generate the sib groups as described by Spelman (2002) is reduced. Fernando and Grossman (1989) evaluated such an MA-BLUP animal model, considering a single marked QTL:

$$y_i = x_i' \beta + v_i^p + v_i^m + u_i + e_i,$$

where y_i is the phenotypic value of the i th animal, v_i^p and v_i^m are the paternal and maternal additive gametic effects of animal i at the QTL, and u_i is the polygenic effect. In general, this MA-BLUP animal model might be difficult to set up for large populations, because only a small fraction of the population is genotyped. Indeed, this is the reason why in the MAS programme of the German Holstein breeders an augmented sire model is used instead (Bennewitz et al., 2003). However, in a bottom-up design most genotyped animals are cows having to be included in the MA-BLUP model. Consequently, a sire model is inappropriate and an animal model has to be applied. Additionally, the size of the Angeln population might enable the use of an MA-BLUP animal model as shown above. As described in **chapter three**, the main challenge during the application of bottom-up MAS is the organisation of DNA collection from the daughters. Therefore, especially for small populations, like the Angeln population, it would be helpful to have markers that show a linkage disequilibrium with the QTL. In these cases the corresponding linkage phases are more or less constant across and have not to be identified for each family separately by bottom-up. Obviously, the most promising situation for a DNA-assisted selection is to have knowledge about the causative mutation. In chapter two, it was emphasised that the DGAT1 K232A mutation and a mutation in the DGAT1 promoter region are known causative mutations affecting milk production traits and also udder health. These two mutations can directly be used for a gene-assisted selection in the Angeln population.

REFERENCES

- Bennewitz, J., N. Reinsch, H. Thomsen, J. Szyda, F. Reinhardt, Z. Liu, Ch. Kühn, A. Tuchscherer, M. Schwerin, C. Weimann, G. Erhardt, and E. Kalm. 2003. Marker assisted selection in German Holstein dairy cattle breeding: outline of the program and marker assisted breeding value estimation. Page 5 in Book of Abstr. 54th Annu. Mtg. EAAP. (Y. van der Honing, ed.) Wageningen Academic Publishers, Wageningen, The Netherlands, p.5.
- Bennewitz, J., N. Reinsch, S. Paul, C. Looft, B. Kaupe, C. Weimann, G. Erhardt, G. Thaller, Ch. Kühn, M. Schwerin, H. Thomsen, F. Reinhardt, R. Reents, and E. Kalm. 2004. The DGAT1 K232A mutation is not solely responsible for the milk production quantitative trait locus on the bovine chromosome 14. *J. Dairy Sci.* 87(2):431–442.
- Bredbacka, P. and Koskinen, T. M. 1999. Microsatellite panels suggested for parentage testing in cattle: informativeness revealed in Finnish Ayrshire and Holstein-Friesian populations. *Agri. Food Sci. Finl.* 8:233–237.
- Cases, S., S. J. Smith, Y.-W. Zheng, H. M. Myers, S. R. Lear, E. Sande, S. Novak, C. Collins, C. B. Welch, A. J. Lusk, S. K. Erickson, and R. V. Farese Jr. 1998. Identification of a gene encoding an acyl CoA:diacylglycerol acyltransferase, a key enzyme in triacylglycerol synthesis. *Proc. Natl. Acad. Sci. USA* 95(22):13018–13023.
- Christensen, L. G., P. Madsen, and J. Petersen. 1982. The influence of incorrect sire-identification on the estimates of genetic parameters and breeding values. *Proc. 2nd World Congr. Genet. Appl. Livest. Prod., Madrid, Spain VII*:200–208.
- Farnir, F., B. Grisart, W. Coppieters, J. Riquet, P. Berzi, N. Cambisano, L. Karim, M. Mni, S. Moisisio, P. Simon, D. Wagenaar, J. Vilkki, and M. Georges. 2002. Simultaneous mining of linkage and linkage disequilibrium to fine map quantitative trait loci in outbred half-sib pedigrees: Revisiting the location of a quantitative trait locus with major effect on milk production on bovine chromosome 14. *Genetics* 161(1):275–287.
- Fernando, R. L. and M. Grossman. 1989. Marker assisted selection using best linear unbiased prediction. *Genet. Sol. Evol.* 21:467–477.
- Geldermann, H., U. Pieper, and W. E. Weber. 1986. Effect of misidentification on the estimation of breeding value and heritability in cattle. *J. Anim. Sci.* 63:1759–1768.
- Grisart, B., W. Coppieters, F. Farnir, L. Karim, C. Ford, P. Berzi, N. Cambisano, M. Mni, S. Reid, P. Simon, R. Spelman, M. Georges, and R. Snell. 2002. Positional candidate cloning of a QTL in dairy cattle: Identification of a missense mutation in the bovine *DGAT1* gene with major effect on milk yield and composition. *Genome Res.* 12(2):222–231.

- Grisart, B., F. Farnir, L. Karim, N. Cambisano, J.-J. Kim, A. Kvasz, M. Mni, P. Simon, J.-M. Frère, W. Coppieters, and M. Georges. 2004. Genetic and functional confirmation of the causality of the *DGATI K232A* quantitative trait nucleotide in affecting milk yield and composition. *Proc. Natl. Acad. Sci. USA*. 101(8):2398–2403.
- Jann, O., E.-M. Prinzenberg, H. Brandt, J. L. Williams, P. Ajmone-Marsan, P. Zaragoza, C. Özbeyaz, and G. Erhardt. 2002. Intragenic haplotypes at the bovine *CSN1S1* locus. *Arch. Tierz.* 45(1):13–21.
- Kühn, Ch., G. Thaller, A. Winter, O. R. P. Bininda-Emonds, B. Kaupe, G. Erhardt, J. Bennewitz, M. Schwerin, and R. Fries. 2004. Evidence for multiple alleles at the *DGATI* locus better explains a quantitative trait locus with major effect on milk fat content in cattle. *Genetics* 167(4):1873–1881.
- Ludwig, E. H., R. W. Mahley, E. Palaoglu, S. Özbayrakçi, M. E. Balestra, I. B. Borecki, T. L. Innerarity, and R. V. Farese Jr. 2002. *DGATI* promoter polymorphism associated with alterations in body mass index, high density lipoprotein levels and blood pressure in Turkish women. *Clin. Genet.* 62(1):68–73.
- Mackinnon, M. J. and M. A. J. Georges. 1998. Marker-assisted preselection of young dairy sires prior to progeny-testing. *Livest. Prod. Sci.* 54(3):229–250.
- Moore, S. S., C. Li, J. Basarab, W. M. Snelling, J. Kneeland, B. Murdoch, C. Hansen, and B. Benkel. 2003. Fine mapping of quantitative trait loci and assessment of positional candidate genes for backfat on bovine chromosome 14 in a commercial line of *Bos taurus*. *J. Anim. Sci.* 81(8):1919–1925.
- Olsen, H. G., S. Lien, M. Gautier, H. Nilsen, A. Roseth, P. R. Berg, K. K. Sundsaasen, M. Svendsen, and T. H. E. Meuwissen. 2005. Mapping of a milk production quantitative trait locus to a 420-kb region on bovine chromosome 6. *Genetics* 169(1):275–283.
- Prinzenberg, E.-M., C. Weimann, H. Brandt, J. Bennewitz, E. Kalm, M. Schwerin, and G. Erhardt. 2003. Polymorphism of the bovine *CSN1S1* promoter: linkage mapping, intragenic haplotypes, and effects on milk production traits. *J. Dairy Sci.* 86(8): 2696–2705.
- Riquet, J., W. Coppieters, N. Cambisano, J.-J. Arranz, P. Berzi, S. K. Davis, B. Grisart, F. Farnir, L. Karim, M. Mni, P. Simon, J. F. Taylor, P. Vanmanshoven, D. Wagenaar, J. E. Womack, and M. Georges. 1999. Fine-mapping of quantitative trait loci by identity by descent in outbred populations: Application to milk production in dairy cattle. *Proc. Natl. Acad. Sci. USA* 96(16):9252–9257.

- Ron, M., Y. Blanc, M. Band, E. Ezra, and J. I. Weller. 1996. Misidentification rate in the Israeli dairy cattle population and its implications for genetic improvement. *J. Dairy Sci.* 79(4):676–681.
- Ron, M., D. Kliger, E. Feldmesser, E. Seroussi, E. Ezra, and J. I. Weller. 2001. Multiple quantitative trait locus analysis of bovine chromosome 6 in the Israeli Holstein population by a daughter design. *Genetics* 159(2):727–735.
- Smith, S. J., S. Cases, D. R. Jensen, H. C. Chen, E. Sande, B. Tow, D. A. Sanan, J. Raber, R. H. Eckel, and R. V. Farese Jr. 2000. Obesity resistance and multiple mechanisms of triglyceride synthesis in mice lacking Dgat. *Nat. Genet.* 25(1):87–90.
- Spelman, R. J. 2002. Utilisation of molecular information in dairy cattle breeding. 7th World Cong. Genet. Appl. Livest. Prod, Communication No. 22-2, Montpellier, France.
- Stella, A., M. M. Lohuis, G. Pagnacco, and G. B. Jansen. 2002. Strategies for continual application of marker-assisted selection in an open nucleus population. *J. Dairy Sci.* 85(9):2358–2367.
- Thaller, G., W. Krämer, A. Winter, B. Kaupe, G. Erhardt, and R. Fries. 2003a. Effects of *DGATI* variants on milk production traits in German cattle breeds. *J. Anim. Sci.* 81(8):1911–1918.
- Thaller, G., Ch. Kühn, A. Winter, G. Ewald, O. Bellmann, J. Wegner, H. Zühlke, and R. Fries. 2003b. *DGATI*, a new positional and functional candidate gene for intramuscular fat deposition in cattle. *Anim. Genet.* 34(5):354–357.
- Van Vleck, L. D. 1970a. Misidentification in estimating the paternal sib correlation. *J. Dairy Sci.* 53:1469–1474.
- Van Vleck, L. D. 1970b. Misidentification and sire evaluation. *J. Dairy Sci.* 53:1697–1702.
- Velmalä, R. J., H. J. Vilkki, K. T. Elo, D. J. de Koning, and A. V. Mäki-Tanila. 1999. A search for quantitative trait loci for milk production traits on chromosome 6 in Finnish Ayrshire cattle. *Anim. Genet.* 30(2):136–143.
- VIT. 2004. Zuchtwertschätzung, Vereinigte Informationssysteme Tierhaltung w.V., Verden/Aller, Germany. <http://www.vit.de>.
- Weller, J. I., Y. Kashi, and M. Soller. 1990. Power of daughter and granddaughter designs for determining linkage between marker loci and quantitative trait loci in dairy cattle. *J. Dairy Sci.* 73(9):2525–2537.
- Weller, J. I., E. Feldmesser, M. Golik, I. Tager-Cohen, R. Domochofsky, O. Alus, E. Ezra, and M. Ron. 2004. Factors affecting incorrect paternity assignment in the Israeli Holstein population. *J. Dairy Sci.* 87(8):2627–2640.

Winter, A., W. Krämer, F. A. O. Werner, S. Kollers, S. Kata, G. Durstewitz, J. Buitkamp, J. E. Womack, G. Thaller, and R. Fries. 2002. Association of a lysine-232/alanine polymorphism in a bovine gene encoding acyl-CoA:diacylglycerol acyltransferase (*DGATI*) with variation at a quantitative trait locus for milk fat content. *Proc. Natl. Acad. Sci. USA* 99(14):9300–9305.

GENERAL SUMMARY

The mapping of quantitative trait loci (QTL) affecting milk production traits in the German Angeln dairy cattle population was the **main objective of this thesis**. Next to the genotyping of microsatellite markers, the *DGATI K232A* mutation, the *DGATI* promoter VNTR, and the *CSN1S1* promoter were analysed. The genotypic information of selected markers were used for the estimation of wrong sire information (WSI). Additionally, the impact of WSI and MSI (missing sire information) on the reliability of estimated breeding values and on the genetic gain were analysed using a deterministic simulation.

In **chapter one** a marker-assisted estimation of WSI with the assistance of 16 microsatellite markers was carried out. Five paternal half-sib families with a total of 805 daughters were genotyped for these highly polymorphic markers on the five bovine chromosomes (BTA) 6, 14, 16, 18, and 27. Blood samples of the daughters and semen samples of the sires were used for the genotyping process. No blood samples of the daughters' dams were available. The allele frequencies and the exclusion probabilities for the involved markers were estimated. The impact of WSI and MSI on the reliability of estimated breeding values and on the genetic gain was investigated using deterministic simulations. Different values for the heritability (0.10, 0.25, and 0.50) and the number of daughters per sire (10, 50, and 100) were chosen. The proportion of WSI was varied in steps from 5 to 30%, and for the MSI the values from 10 up to 40% were taken. The estimated proportion of WSI was 7% and the proportion of MSI was 10%. The results of the deterministic simulations showed that WSI and MSI had an effect on the reliability of estimated breeding values and that the combined impact of WSI and MSI was relatively large on the genetic gain. The impact of WSI on the efficiency was around 1.4 times more harmful than the impact of MSI.

The calculation of the frequencies and of the effects of the alleles at the *DGATI K232A* mutation and of the *DGATI* promoter VNTR on BTA14 was the main objectives of **the second chapter**. Furthermore, the estimation of the allele frequencies and the calculation of the effects of the alleles at the *CSN1S1* promoter on BTA6 were investigated. The following milk production traits were included in the statistical analysis: milk yield, fat yield and content, protein yield and content, milk energy yield and content, lactose yield and content as well as somatic cell score. As phenotypic data yield deviations were used. The allele frequency of the lysine variant at the *DGATI K232A* mutation was 0.61. Three sires were homozygous for the lysine variant. Furthermore, it was shown that the lysine variant showed

significant effects for the main part of the included milk production traits. Six alleles at the VNTR polymorphism (variable number of tandem repeats) in the *DGATI* promoter region were found. One VNTR allele showed significant effects for the main part of the involved milk production traits. In some traits the effects of the lysine variant were intensified through the effects of the *DGATI* promoter VNTR allele.

The segregation of four alleles at the *CSN1S1* promoter were investigated. The *CSN1S1* alleles 2 and 3 showed significant effects for the yield traits milk, fat, protein, lactose, and milk energy yield. No significant results were observed for the milk content traits and somatic cell score.

The mapping of QTL affecting milk production traits (milk, fat, protein, lactose, and milk energy yield, fat, protein, lactose, and milk energy content as well as somatic cell score) in the German Angeln dairy cattle population was the objective of **the third chapter**. Some of these traits (e.g., milk yield) are rather complex and the additional information of QTL for related traits shows a clearer physiological background and might help to explain the milk production QTL better. Following a daughter design, five paternal half-sib families with a total of 805 daughters were genotyped for 43 markers, including the *DGATI* K232A mutation. The markers were distributed over the five chromosomes 6, 14, 16, 18, and 27 with an average marker distance of 20 cM. For the statistical analysis three weighted regression models were applied. Chromosomewise significant QTL were identified on BTA6 for lactose and protein content. Segregating QTL affecting the traits lactose content and yield, protein yield, and milk energy yield were identified on BTA18. No QTL were segregating for the other traits and on the other chromosomes, respectively.

In **chapter four** additional aspects regarding the findings of the previous three chapters are discussed.

ZUSAMMENFASSUNG

Die Kartierung von QTL (Gene für quantitative Merkmale) mit einem Einfluss auf die verschiedenen Milchproduktionsmerkmale in der Deutschen Angler Rind-Population war das **Hauptthema der vorliegenden Arbeit**. Neben der Typisierung von Mikrosatelliten wurden die *DGATI-K232A*-Mutation, der *DGATI*-Promotor-VNTR und der *CSN1S1*-Promotor analysiert. Die genotypischen Informationen einiger hoch polymorpher Marker wurde für eine Schätzung des Anteils an falschen Abstammungen (Wrong Sire Information) genutzt. Des Weiteren wurde der Einfluss von falschen und fehlenden Abstammungen (Missing Sire Information) auf die Sicherheit der geschätzten Zuchtwerte und auf den Zuchtfortschritt mittels deterministischer Simulationen ermittelt.

Im **ersten Kapitel** wurde eine markergestützte Schätzung des Anteils an falscher Abstammung mit der Hilfe von 16 hoch polymorphen Mikrosatelliten durchgeführt. Es erfolgte eine Typisierung von fünf väterlichen Halbgeschwisterfamilien (mit insgesamt 805 Töchtern) auf den Chromosomen 6, 14, 16, 18 und 27. Für die genotypischen Untersuchungen wurden Blutproben der Töchter und Spermabproben der Väter genutzt. Von den Müttern waren keine Blutproben vorhanden. Zusätzlich wurden Allelfrequenzen und Ausschlusswahrscheinlichkeiten (Exclusion Probabilities) der untersuchten Mikrosatelliten geschätzt. Der Einfluss von falschen und fehlenden Abstammungen auf die Sicherheit der geschätzten Zuchtwerte und auf den Zuchtfortschritt wurden mittels deterministischer Simulationen festgestellt. Ziel war es, eine möglichst realistische Situation in der Angler-Population wiederzugeben. Aus diesem Grund wurden verschiedene Werte für die Heritabilität ($h^2 = 0,10; 0,25$ und $0,50$) sowie unterschiedliche Größen der Töchtergruppen pro Vater (10, 50 und 100 Töchter) gewählt. Der Anteil an falschen Abstammungen wurde in verschiedenen Schritten zwischen 5 und 30 % festgelegt. Der Anteil an fehlender Abstammung variierte zwischen 10 und 40 %. Der geschätzte Anteil an falschen Abstammungen beträgt 7 % und der Anteil von fehlenden Abstammungen liegt bei 10 % in der Deutschen Angler Rind-Population. Die Simulationen zeigten, dass sowohl falsche als auch fehlende Abstammungen einen großen Einfluss auf die Sicherheit der geschätzten Zuchtwerte besitzen. Das simultane Auftreten von beiden Pedigreefehlern führte zu einer Verstärkung des Effektes. Des Weiteren konnte festgestellt werden, dass der Einfluss von falschen Abstammungen um ein 1,4-faches größer ist als der Einfluss von fehlenden Abstammungen.

Die Berechnung der Frequenzen und der Substitutionseffekte der Allele der *DGATI-K232A*-Mutation und des *DGATI*-Promotors-VNTR auf dem bovinen Chromosom 14 waren die Hauptpunkte des **zweiten Kapitels**. Zusätzlich wurden Allelfrequenzen und Substitutionseffekte der Allele des *CSN1S1*-Promotors auf Chromosom 6 berechnet. Die folgenden Milchproduktionsmerkmale wurden untersucht: Milch-, Fett-, Protein-, Laktose- und Milchenergiemenge, Fett-, Protein-, Laktose- und Milchenergiegehalt, sowie Zellzahl. Yield deviation wurden als phänotypische Datengrundlage verwendet. Die Allelfrequenz der Lysinvariante beträgt bei den Anglern 0,61. Drei der fünf Väter waren homozygot für die Lysinvariante und die beiden anderen waren heterozygot an der *DGATI-K232A*-Mutation. Für den größten Teil der untersuchten Milchmerkmale konnten signifikante Effekte für die Lysinvariante festgestellt werden. In der *DGATI*-Promotor-Region konnten sechs unterschiedliche Allele des VNTR-Polymorphismus (Variable Number of Tandem Repeats) typisiert werden. Auch in diesem Fall konnten für die meisten Merkmale signifikante Effekte eines VNTR-Allels festgestellt werden. Des Weiteren konnte eine Intensivierung der Lysineffekte durch das VNTR-Allel beobachtet werden.

Am *CSN1S1*-Promotor konnten vier unterschiedliche Allele festgestellt werden. Die Allele 2 und 3 zeigten signifikante Effekte für die Milchmengenmerkmale Milch-, Fett-, Protein-, Laktose- und Milchenergiemenge. Für die Gehaltsmerkmale und Zellzahl konnten keine signifikanten Effekte beobachtet werden.

Die Kartierungsergebnisse von QTL mit Einfluss auf die Milchproduktionsmerkmale (Milch-, Fett-, Protein-, Laktose- und Milchenergiemenge, Fett-, Protein-, Laktose- und Energiegehalt sowie Zellzahl) in der Deutschen Angler Rind-Population wurden im **dritten Kapitel** vorgestellt. Einige der Mengenmerkmale, z.B. Milchmenge, zeichnen sich durch einen komplexen, physiologischen Charakter aus, der durch zusätzliche QTL-Informationen möglicherweise besser verstanden werden kann. Einem Daughter Design (Töchtervergleich) folgend wurden fünf verschiedene väterliche Halbgeschwisterfamilien (mit insgesamt 805 Töchtern) mit 43 Markern typisiert, inklusive der *DGATI-K232A*-Mutation. Die verwendeten Marker waren auf den fünf Chromosomen 6, 14, 16, 18 und 27 verteilt. Die durchschnittliche Markerdistanz betrug 20 cM. Drei gewichtete Regressionsanalysen wurden für die einzelnen Merkmale durchgeführt. Chromosomenweite signifikante QTL konnten auf Chromosom 6 für die Merkmale Laktose- und Proteingehalt festgestellt werden. Des Weiteren wurden segregierende QTL auf Chromosom 18 für die Merkmale Laktosegehalt und -menge,

Proteinmenge und Milchenergiemenge festgestellt. Es konnten keine signifikanten QTL für die anderen Merkmale sowie für die anderen Chromosomen ermittelt werden.

Im **vierten Kapitel** erfolgte eine abschließende Diskussion der Ergebnisse aus den vorherigen drei Kapiteln.

APPENDIX I

The PCR (polymerase chain reaction) conditions and the individual reagent mixes for the used microsatellite markers (including the *DGATI K232A* mutation) are described in the following chapter. The PCR for the used microsatellite markers were done on a MJ Research PTC-200 thermocycler (Global Medical Instrumentation Inc., Minnesota). The used total volume of each reaction was 10 μ l and the DNA was aliquoted in a dried condition in 96-deep well plates. The PCR conditions varied mainly in the annealing temperature and in the number of cycles for the different markers. The first denaturing step at 94°C was done for 3 min and the final extension time (72°C) was set between 10 and 30 min, depending on the used markers. Each cycle includes the following steps: a denaturing step at 94 °C for 20 sec, a primer-specific annealing step (between 54 °C and 64 °C) for 30 sec, and an extension step at 72°C for 30 sec. The number of cycles depends on the used marker (between 27 and 45 cycles). The standard reagent mix for 10- μ l volume is presented in the following:

3	μ l genomic DNA
50	mM KCl ¹
1.5	mM MgCl ₂
20	mM Tris-HCl (pH 8,4) ¹
0.2 – 0.5	μ M of each primer ²
200	μ M dNTPs
0.2 – 0.4	Units <i>Taq</i> -polymerase ²

The superscript 1 denotes the ingredients of the 10X PCR buffer (Invitrogen Corporation, Germany) and the superscript 2 denotes the PCR components which varied for the different primer pairs.

APPENDIX II

Table 1: Used and tested multiplex-combinations, chromosomes (BTA), dye, and PCR conditions of the used microsatellite markers

Multiplex	Marker	BTA	Dye	Primer ¹ (μ l)	Taq (units)	Buffer	T _A (°C) ²	Number of cycles
ANG1_1	BM1329	06	hex	0.2	0.3	1.3	58°C	35
	HUJ614	16	hex					
	BMS719	16	hex					
ANG2_1	BM3507	27	fam	0.2	0.3	1.3	55°C	35
	CSSM043	27	fam					
ANG3_1	RM209	27	fam	0.2	0.3	1.3	58°C	35
	INRA027	27	fam					
ANG4_1	BM1311	16	fam	0.2	0.3	1.3	55°C	35
	BM143	06	fam					
ANG5_1	BM4528	06	tet	0.2	0.3	1.3	55°C	35
	INRA048	16	tet					
	INRA134	27	tet					
ANG6_1	CSSM028	16	fam	0.2	0.3	1.3	55°C	35
	TGLA179	27	fam					
	BM415	06	hex					
ANG7_1	TGLA037	06	hex	0.2	0.3	1.3	55°C	35
	HUJ625	16	tet					
ANG8_1	INRA133	06	hex	0.2	0.3	1.0	55°C	35
	BMS1675	27	fam					
ANG9_1	BL1038	06	hex	0.2	0.3	1.0	55°C	35
	MGTG1	16	tet					
M3_1	BM7109	18	fam	0.2	0.4	1.0	55°C	35
	ILSTS002	18	hex	0.3				
	TGLA227	18	fam	0.2				
M3_4	DIK082	06	hex	0.25	0.4	1.0	55°C	39
	CSSM066	14	hex					
M3_10	BM2078	18	fam	0.3	0.4	1.0	55°C	45
	CSN3	06	fam	0.2				
	BMS2639	18	tet	0.2				

¹ Concentration 10 μ mol/l per primer.

² Annealing temperature.

Table 2: Used and tested microsatellite markers with single amplification, chromosomes (BTA), dye, and PCR conditions of the used microsatellite markers

Marker	BTA	Dye	Primer ¹ (μ l)	Taq (units)	Buffer	T _A (°C) ²	Number of cycles
BMC4203	06	fam	0.2	0.4	1.0	55°C	27
BP7	06	hex	0.2	0.3	1.3	55°C	40
<i>DGATI</i>	14	fam	0.2	0.4	1.0	56°C	35
ILSTS039	14	hex	0.2	0.4	1.3	58°C	32
ILSTS011	14	tet	0.2	0.3	1.0	55°C	30
BMS1678	14	hex	0.2	0.3	1.0	55°C	35
BM302	14	tet	0.2	0.3	1.0	55°C	32
BM6425	14	fam	0.2	0.3	1.0	55°C	35
RM180	14	hex	0.5	0.4	1.2	64°C	35
BMS1899	14	tet	0.2	0.2	1.0	55°C	30
BM4513	14	fam	0.2	0.3	1.0	56°C	35
BMC1207	14	tet	0.2	0.3	1.0	55°C	30
BMS2785	18	hex	0.2	0.2	1.0	57°C	35
BMS833	18	hex	0.2	0.2	1.0	54°C	35
BM6507	18	hex	0.2	0.2	1.0	55°C	32
BM203	27	fam	0.2	0.3	1.3	62°C	35

¹ Concentration 10 μ mol/l per primer

² Annealing temperature

APPENDIX III

Table 3: Primer sequences of the used microsatellite markers with marker name and their respective chromosome (BTA)

Marker name	BTA	Forward primer (5'-3')	Reverse primer (5'-3')
AJ318490 (<i>DGAT1</i> promoter)	14	TCA GGA TCC AGA GGT ACC AG	GTG TCT TGG GGT CCA AGG TTG ATA CAG ¹
AY065621 (<i>DGAT1K232A</i>)	14	CCT GAC TGG CCG CCT GCC GCT TGC TCG TAG	GCG GGG GAA GTT GAG CTC GTA G
BL1038	06	GTG TCT TGG CAA GCT AGA GTC AGA CAC ¹	GCA AAA GTC TAG GTG AAA TGC C
BM143	06	ACC TGG GAA GCC TCC ATA TC	GTG TCT TCT GCA GGC AGA TTC TTT ATC G ¹
BM302	14	GAA TTC CCA TCA CTC TCT CAG C	GTT CTC CAT TGA ACC AAC TTC A
BM415	06	GTG TCT TTG GCT ACA GCC CTT CTG GTT ¹	GAG AGC TAA TCA CCA ACA GCA AGA
BM1311	16	GTG TCT TGA CTG AGC GCA GGC ATC G	AGC CAT ACA TAG GTG GGA GCT GAA
BM1329	06	TTG TTT AGG CAA GTC CAA AGT C	AAC ACC GCA GCT TCA TCC
BM2078	18	CCC AAA AGA AGC CAG GAA G	TCA GAG TTT GGG GTC CTC AG
BM3507	27	GCC CAA AGA AAG AAG TAT GTG C	TAG TGC GGA GTC AGT CAT GTG
BM4513	14	GTG TCT TCA TGC ACT TTT CCT TCT GGT TC ¹	TCA ACT CAG CAA TTC AGT ACA TCA
BM4528	06	GTG TCT TTT CAT TTA TCC TAG ACT CTA AAT GC ¹	TGA GGA ACA GGT ATA GGA ATA TTG G
BM6425	14	GTG TCT TAT GTG AAC CTG GGT CTC CTG ¹	TGC AAT GGC ATG GAA AAA AG
BM6507	18	ACT TAG CAC AAT GCC CTC TAG G	ATG TTA TTC CAT CAG GAG GAG C
BM7109	18	CAG GTA AAA GAG CGG CTT TG	CAG CTT CAT GCC CTA GAA GG
BMC1207	14	ACC AAC AAG TCT GAA TCT TCA TT	GGG TGG AAT AGT CAG TCC CA
BMC4203	06	GTA TGA GTG CCC TTG TTC AC	AGG AGG AAA TGG GCT AAC TA
BMS719	16	GTG TCT TAA GTG CAC GCT AAC ACG TTG ¹	CCT CCC TCT CCC TCT GTT TCT
BMS833	18	CCA TGA GGA CTG CCA AAA AT	TAA AGG CCT CTC TTG AAA TTC C
BMS1675	27	GTG TCT TCA TTA GAA AGC TGA TTG GAG GG ¹	TAA TAA TCA GTG CCG CTC CC
BMS1678	14	GTG TCT TAT AGC TGA CAT CCA CTG GGC ¹	TCT TCT CTG CAC TTT GGT TGC
BMS1899	14	TCC TAT TCA TCC TGT TAT TGC C	GGA GTC AGA CAT GAC AAG TGA C

¹ Primers with a so-called pigtail (GTG TCT).

Table 3a: Continuation of Table 3

Marker Name	BTA	Forward Primer (5'-3')	Reverse Primer (5'-3')
BMS2639	18	ATA TCG TTT TCA GAT TTC TTT TGC	GAG AGA TAA ATT GGG AGT TTG AGA
BMS2785	18	TTA GGA GAT AAG GCA GAA GCC CGC TCA	AAA TGG CAA TCA GTC GGA CAC ACG C
BP7	06	GAC CTT TTC ACT GCC CTC TG	TTT ATT TCT GAG TCT TTG GGG C
CSN3	06	CCA ACA TAT AAA CCC AGG AAT CC	GAC ATA CAA TAC ACA AGC ATA C
CSSM028	16	GTGTCTTCACCTAAGGAGTTGAAGAATGATAACAG ¹	TCA TAT TTA CTG AAG ATC CCT TCT AAT GAG
CSSM043	27	AAA ACT CTG GGA ACT TGA AAA CTA	GTT ACA AAT TTA AGA GAC AGA GTT
CSSM066	14	ACA CAA ATC CTT TCT GCC AGC TGA	AAT TTA ATG CAC TGA GGA GCT TGG
DIK082	06	CCC ACT CTG TCT CCA GTT TG	TAT CCT GAG AAA AGC TGC TAG A
HUJ614	16	GTG TCT TCC AGG CAT GGT GAA GTC GAA AAG ¹	CAC AGT ACA GGC TGC TCT GGT TGA A
HUJ625	16	GTG TCT TGA GGT CAC ATA CCC ATC AAG C ¹	AGC AGC ATG AAG AGA GTC CC
ILSTS002	18	TCT ATA CAC ATG TGC TGT GC	CTT AGG GGT GAA GTG ACA CG
ILSTS011	14	GCT TGC TAC ATG GAA AGT GC	CTA AAA TGC AGA GCC CTA CC
ILSTS039	14	CCT AAT GAC TAC CAA CAG GG	TCC ATG GAA TCA CAA AGA GC
INRA027	27	CTC CCC ACT TAG GAA CTC TGT ATC	GTG TCT TCA CTG CAT CCC TCC CCA CTA AC ¹
INRA048	16	GTG TCT TAA ACT GTC CCT CAG TAA ACA AGT CG ¹	AAG CTA AAG TAG CAG GGA AGG GC
INRA133	06	GTG TCT TGG GTT GTC TCT GCT GTT AAA TTG ¹	TAT GAG AAA GTG CAT GCA AAT GA
INRA134	27	CCA GGT GGG AAT AAT GTC TCC	TTG GGA GCC TGT GGT TTA TC
MGTG1	16	GTGTCTTTGCATAACAAAGAAAAGTAGCTGAG ¹	TTA GTT TGT CCC TAG TGC TCT CAT G
RM180	14	TGG CCA AGA CAT CTG CCA TTC C	GGA GTC TGG TGG GTT ACA GTC C
RM209	27	GTA GAA GTT AGT GAC TGT CAT CC	CCT CAG AGC CCC ATA CAT TTC C
TGLA037	06	GTG TCT TCA TTC CAA TCC CCT ATC CTG AG ¹	TTG AAT GAT TCT ATG AAG ACC TCT A
TGLA179	27	CTT TAA TCA GCA CAC AGC TTC CCA	GTG TCT TAT ATG TGC TAG AAG TTT GGT CAA CC ¹
TGLA227	18	CGA ATT CCA AAT CTG TTA ATT TGC T	ACA GAC AGA AAC TCA ATG AAA GCA

¹ Primers with a so-called pigtail (GTG TCT).

APPENDIX IV

This chapter presents the information contents along the investigated chromosomes. The information content for each cM is defined as the average of the absolute values of the daughters QTL transition probabilities at the corresponding cM.

$$\frac{1}{N} \sum_{i=1}^N |1 - 2p_i|$$

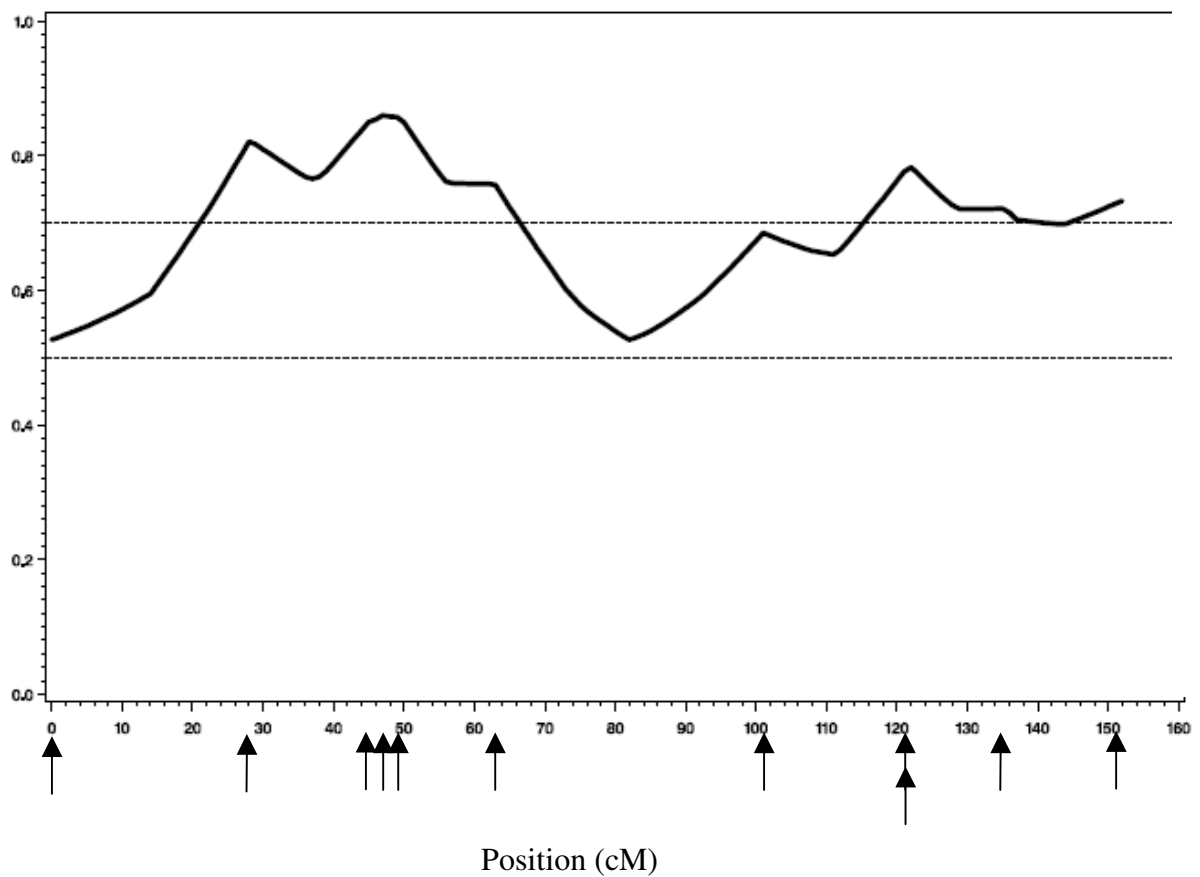


Figure 1: Information content along BTA6. The arrows indicate the positions of the genetic markers.

$$\frac{1}{N} \sum_{i=1}^N |1 - 2p_i|$$

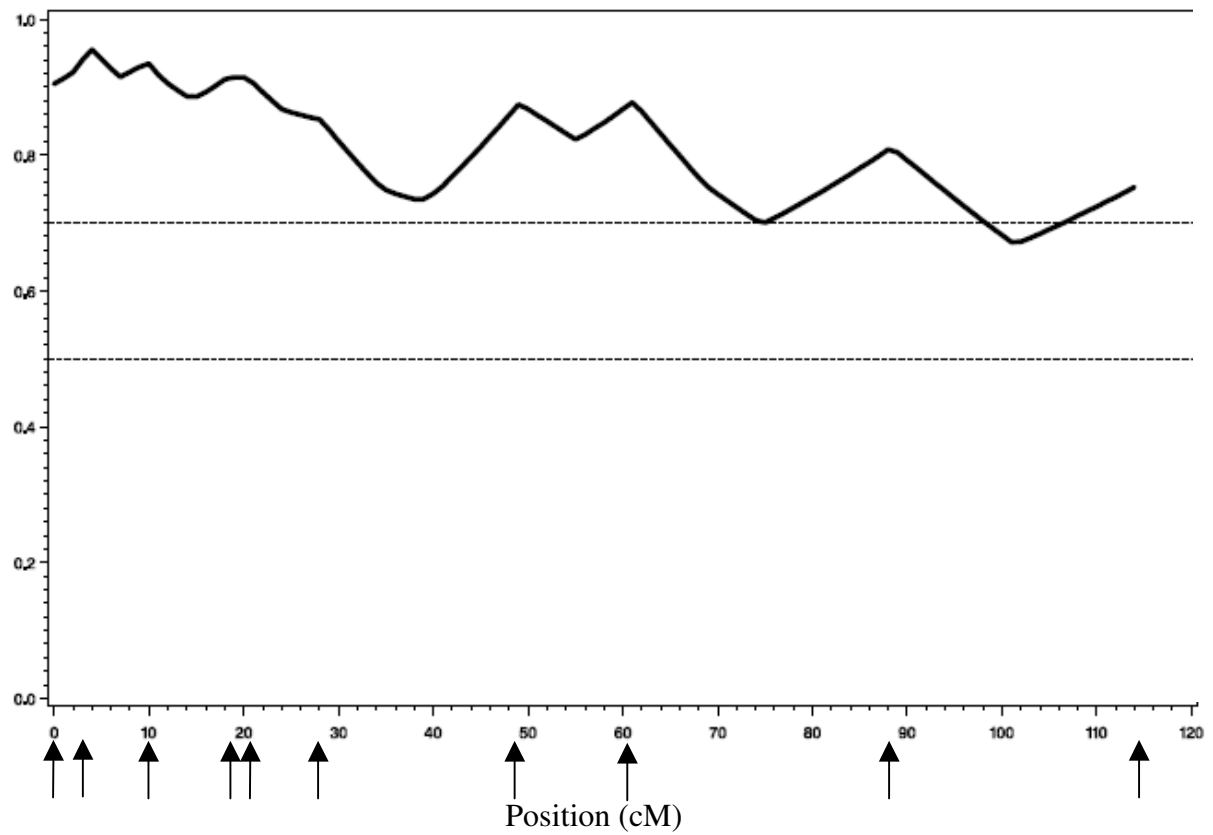


Figure 2: Information content along BTA14. The arrows indicate the positions of the genetic markers.

$$\frac{1}{N} \sum_{i=1}^N |1 - 2p_i|$$

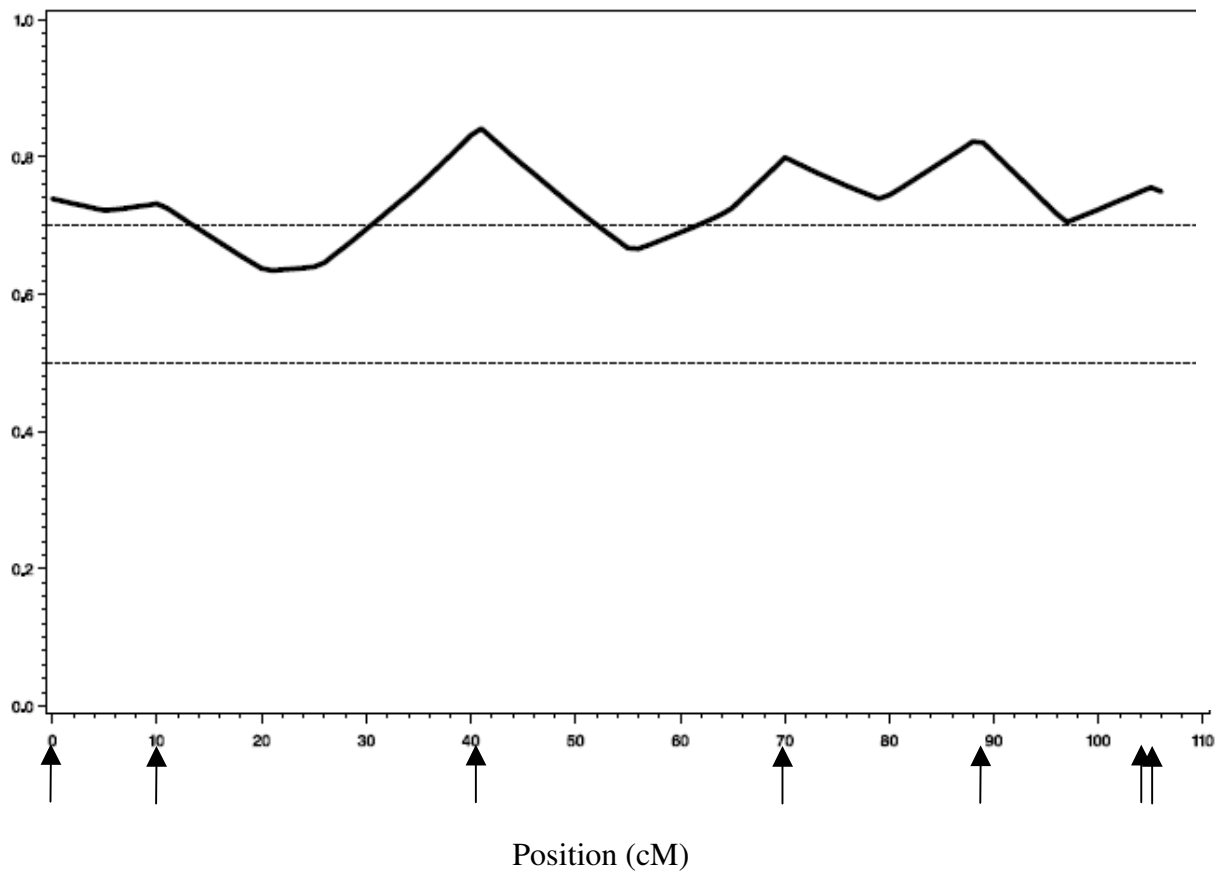


Figure 3: Information content along BTA16. The arrows indicate the positions of the genetic markers.

$$\frac{1}{N} \sum_{i=1}^N |1 - 2p_i|$$

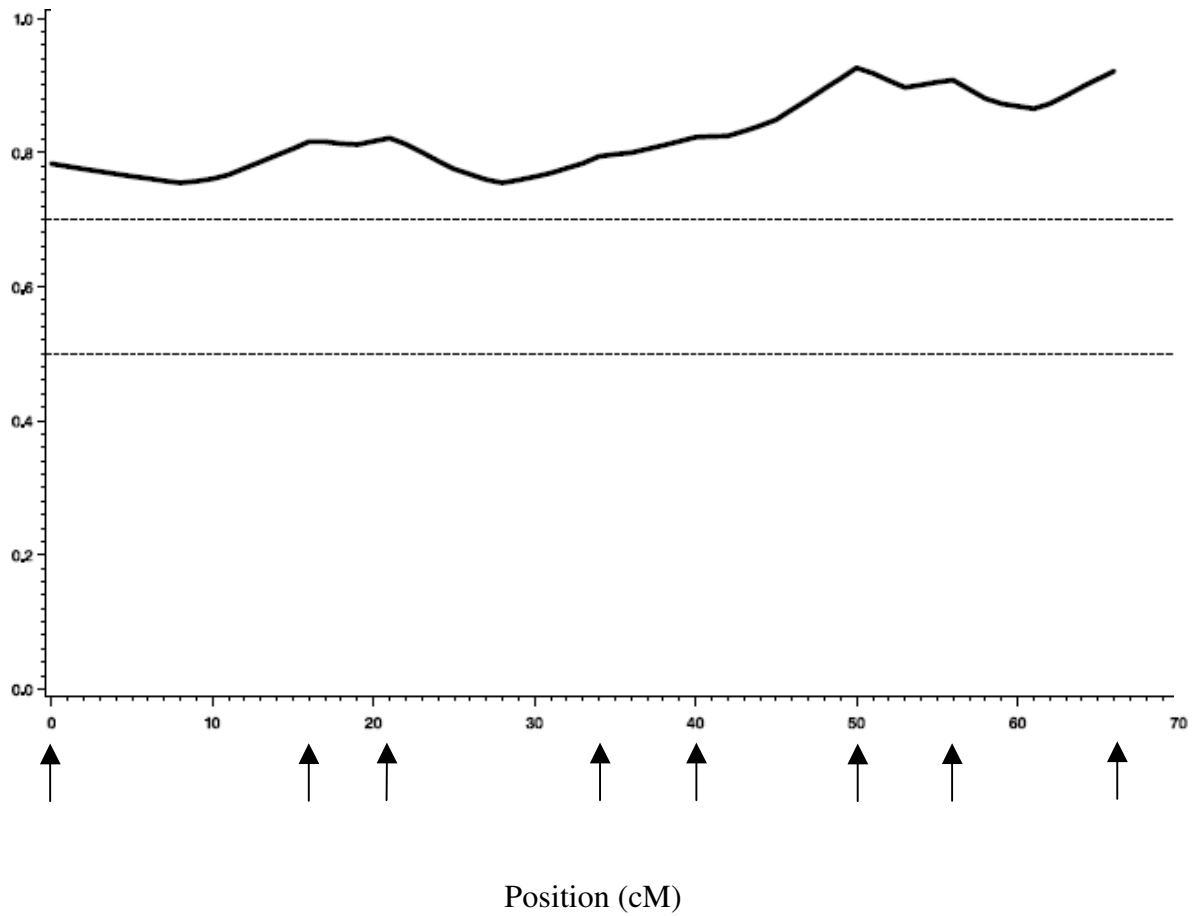


Figure 4: Information content along BTA18. The arrows indicate the positions of the genetic markers.

$$\frac{1}{N} \sum_{i=1}^N |1 - 2p_i|$$

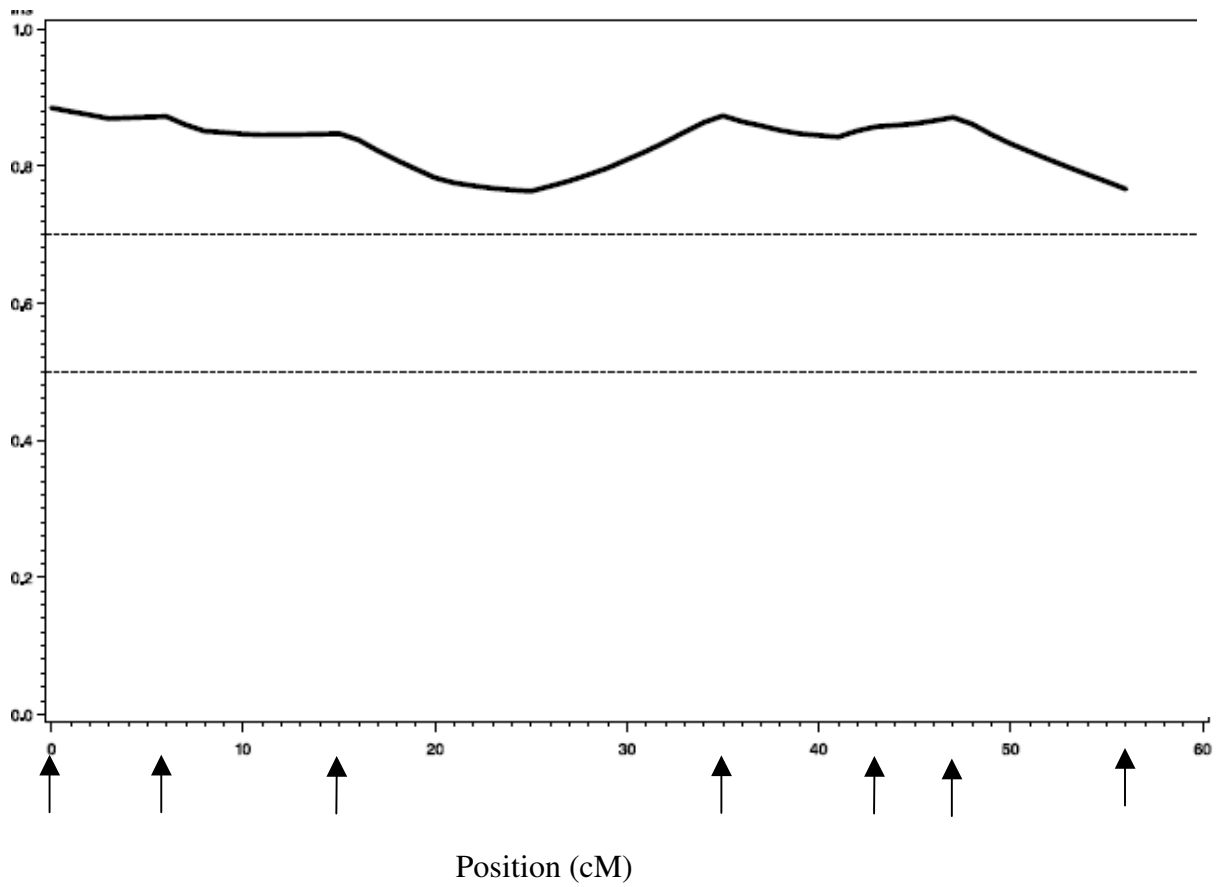


Figure 5: Information content along BTA27. The arrows indicate the positions of the genetic markers.

APPENDIX V

Table 4: Marker positions (cM), recombination rates (θ), and the corresponding LOD scores from the build and twopoint analysis of Crimap, respectively, on chromosome 6

Marker	Position (cM)	Recombination rate (θ)	LOD score
INRA133	0.0	-	-
BM1329	28.1	0.24	9.88
BM143	45.0	0.14	18.95
TGLA037	47.3	0.02	13.21
DIK082	49.4	0.03	27.11
BM4528	62.9	0.12	30.73
BM415	101.0	0.50	0.00
<i>CSN3</i>	121.7	0.34	1.93
<i>CSN1S1</i>	121.7	0.00	18.36
BP7	135.4	0.06	12.47
BMC4203	152.2	0.13	11.68

- Missing, because of the consideration of the interval between two loci.

Table 5: Marker positions (cM), recombination rates (θ), and the corresponding LOD scores from the build and twopoint analysis, respectively, on chromosome 14

Marker	Position (cM)	Recombination rate (θ)	LOD score
<i>DGATI</i>	0.0	-	-
ILSTS039	3.9	0.04	38.16
CSSM066	9.9	0.06	72.76
BMS1678	18.7	0.11	27.23
ILSTS011	20.2	0.02	67.51
RM180	28.2	0.06	29.95
BM302	49.1	0.16	29.06
BMS1899	61.1	0.10	53.51
BM4513	88.3	0.25	15.34
BM6425	114.4	0.27	11.12

- Missing, because of the consideration of the interval between two loci.

Table 6: Marker positions (cM), recombination rates (θ), and LOD scores from the build and twopoint analysis of Crimap, respectively, on chromosome 16

Marker	Position (cM)	Recombination rate (θ)	LOD score
MGTG1	0.0	-	-
HUJ614	10.3	0.10	13.78
BM1311	40.8	0.27	7.91
CSSM028	70.0	0.25	15.82
INRA048	88.5	0.18	24.43
HUJ625	105.3	0.17	33.55
BMS719	106.3	0.01	97.92

- Missing, because of the consideration of the interval between two loci.

Table 7: Marker positions (cM), recombination rates (θ), and LOD scores from the build and twopoint analysis of Crimap, respectively, on chromosome 18

Marker	Position (cM)	Recombination rate (θ)	LOD score
BM7109	0.0	-	-
ILSTS002	16.3	0.16	12.98
BMS2639	21.2	0.06	24.76
BMS833	34.2	0.25	3.84
BMS2785	40.2	0.00	0.00
BM2078	50.1	0.06	25.59
BM6507	55.9	0.06	43.51
TGLA227	66.7	0.12	35.75

- Missing, because of the consideration of the interval between two loci.

Table 8: Marker positions (cM), recombination rates (θ), and LOD scores from the build and twopoint analysis of Crimap, respectively, on chromosome 27

Marker	Position (cM)	Recombination rate (θ)	LOD score
BM3507	0.0	-	-
TGLA179	6.0	0.06	24.21
RM209	15.4	0.05	16.05
CSSM043	34.8	0.16	17.54
INRA134	42.5	0.08	11.08
INRA027	47.3	0.04	11.67
BM203	56.7	0.06	8.95

- Missing, because of the consideration of the interval between two loci.

APPENDIX VI

In the following the test statistics for the ten milk production traits milk, fat, protein, lactose, and milk energy yield, fat, protein, lactose, and milk energy content, as well as somatic cell score are presented.

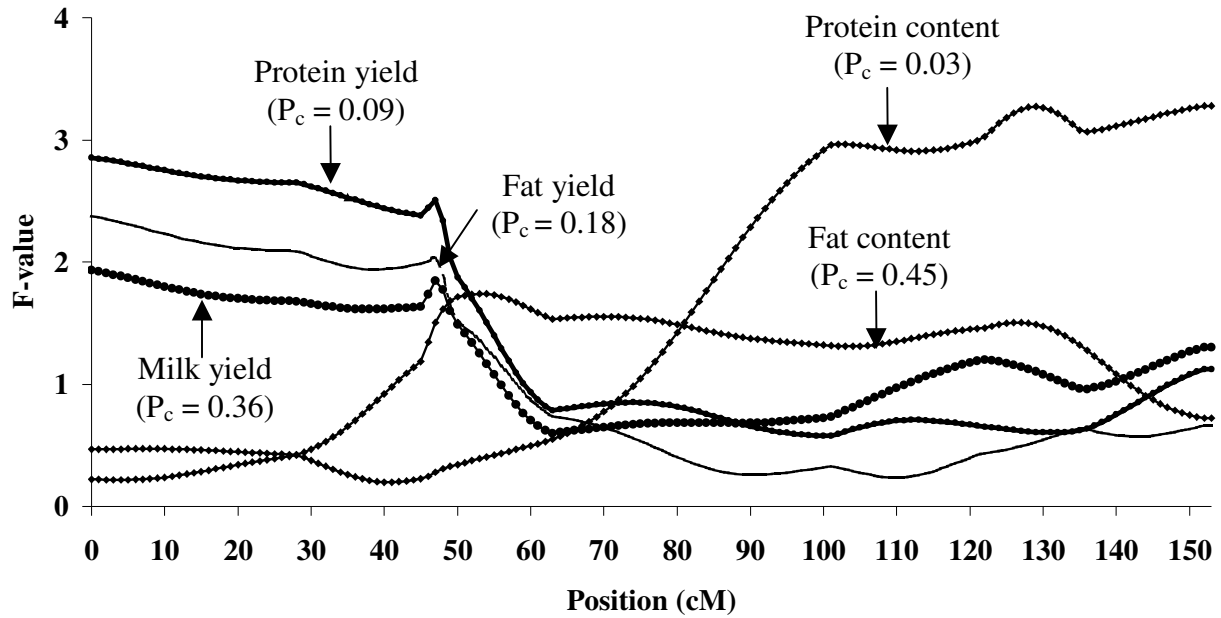


Figure 6: Test statistics for the five traits protein yield and content, fat yield and content and milk yield on BTA6, results of the multimarker regression model included *DGATI K232A* (Chapter three).

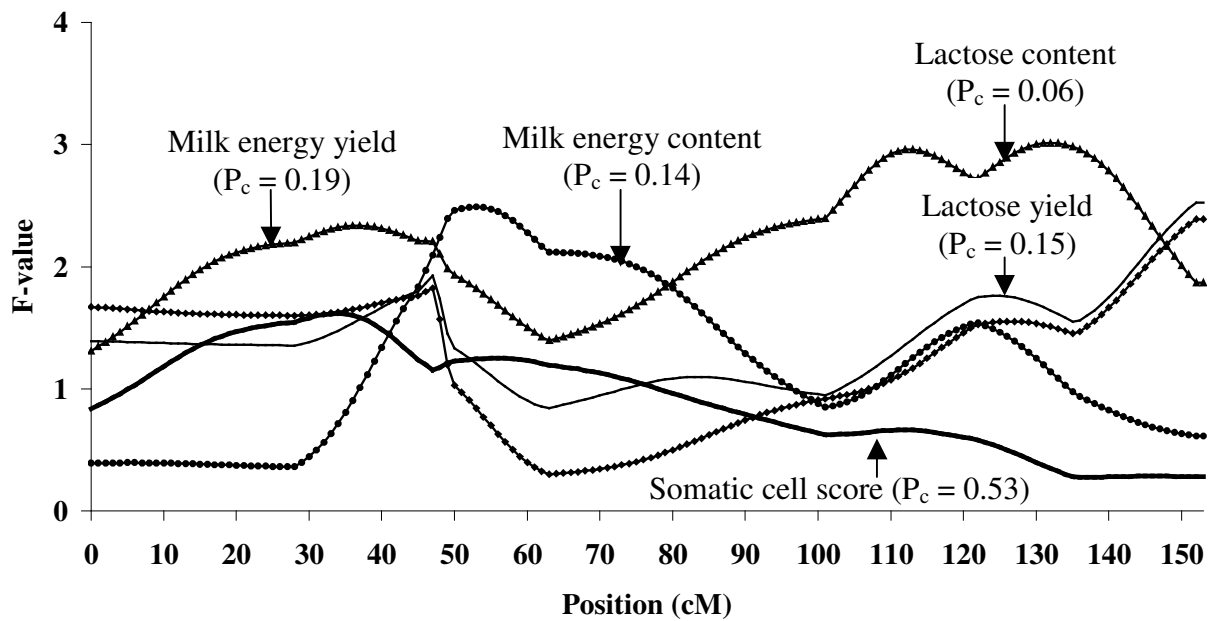


Figure 7: Test statistics for the traits milk energy content and lactose yield as a result of the multimarker regression model included *DGATI K232A*, and the traits milk energy yield, lactose content, and somatic cell score as a result of the classical multimarker regression model (Chapter three) on BTA6.

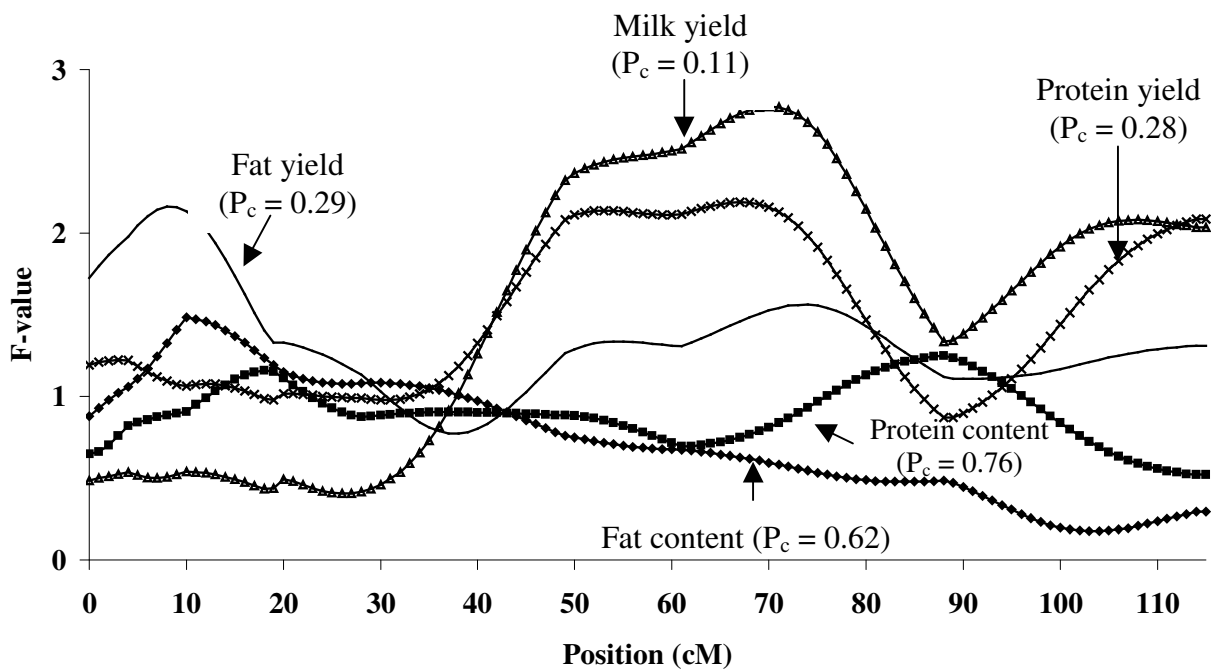


Figure 8: Test statistics for the five traits protein yield and content, fat yield and content and milk yield on BTA14, results of the multimarker regression model included *DGATI K232A* (Chapter three).

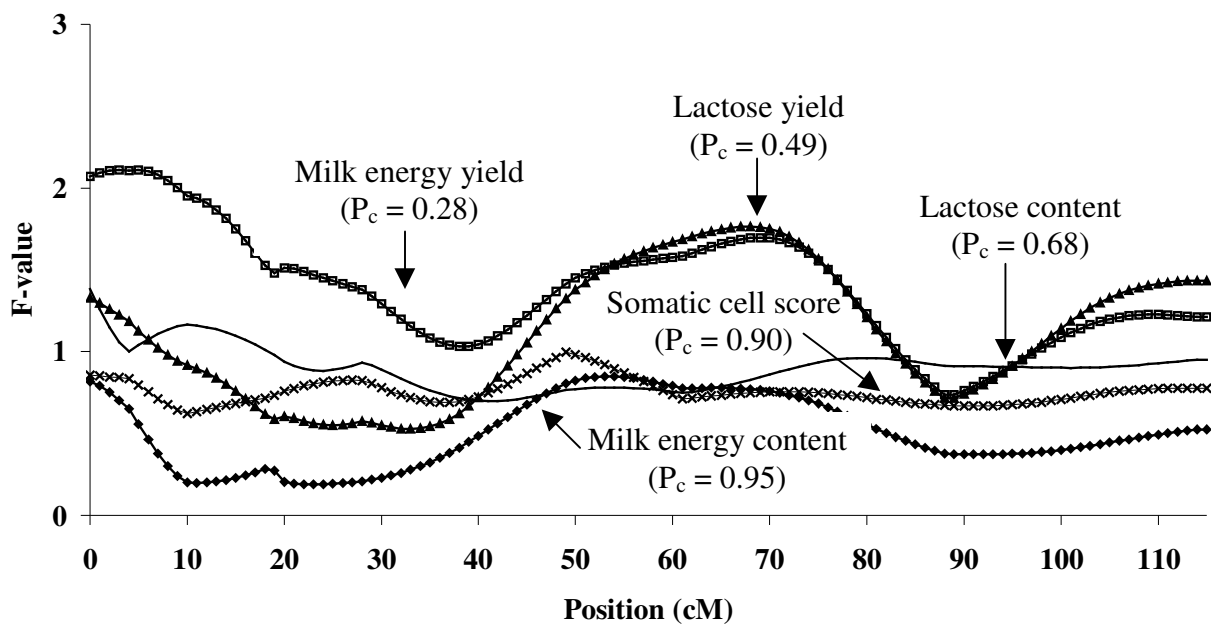


Figure 9: Test statistics for the traits milk energy content and lactose yield as a result of the multimarker regression model included *DGATI K232A*, and the traits milk energy yield, lactose content, and somatic cell score as a result of the classical multimarker regression model (Chapter three) on BTA14.

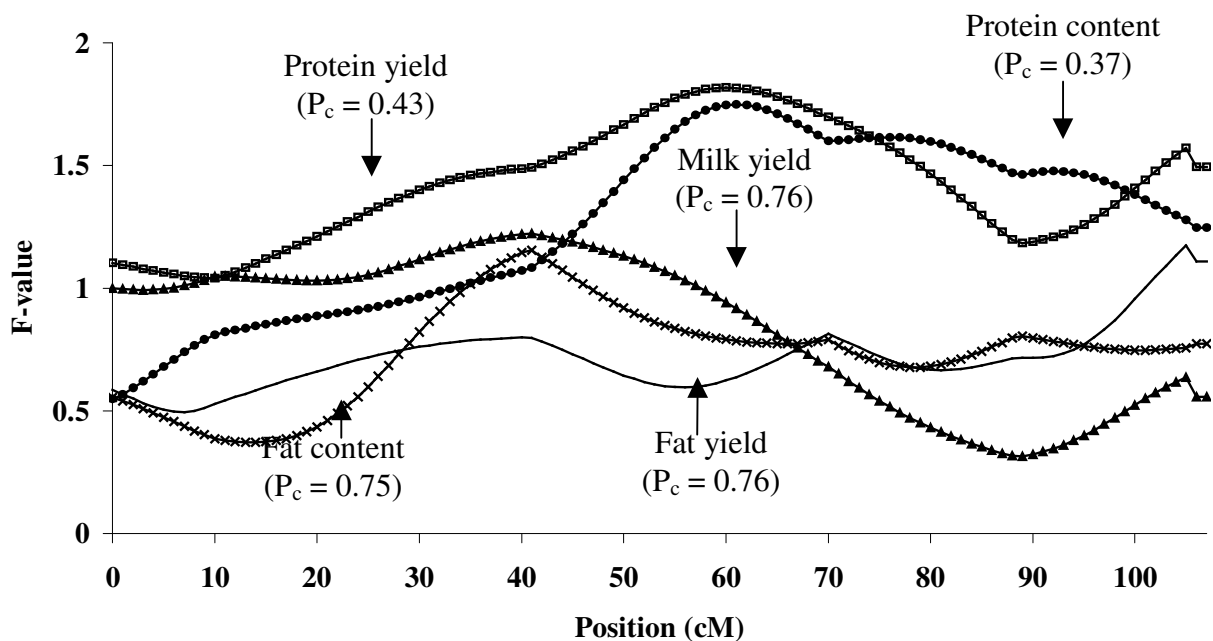


Figure 10: Test statistics for the five traits protein yield and content, fat yield and content, and milk yield on BTA16, results of the multimarker regression model included *DGATI K232A* (Chapter three).

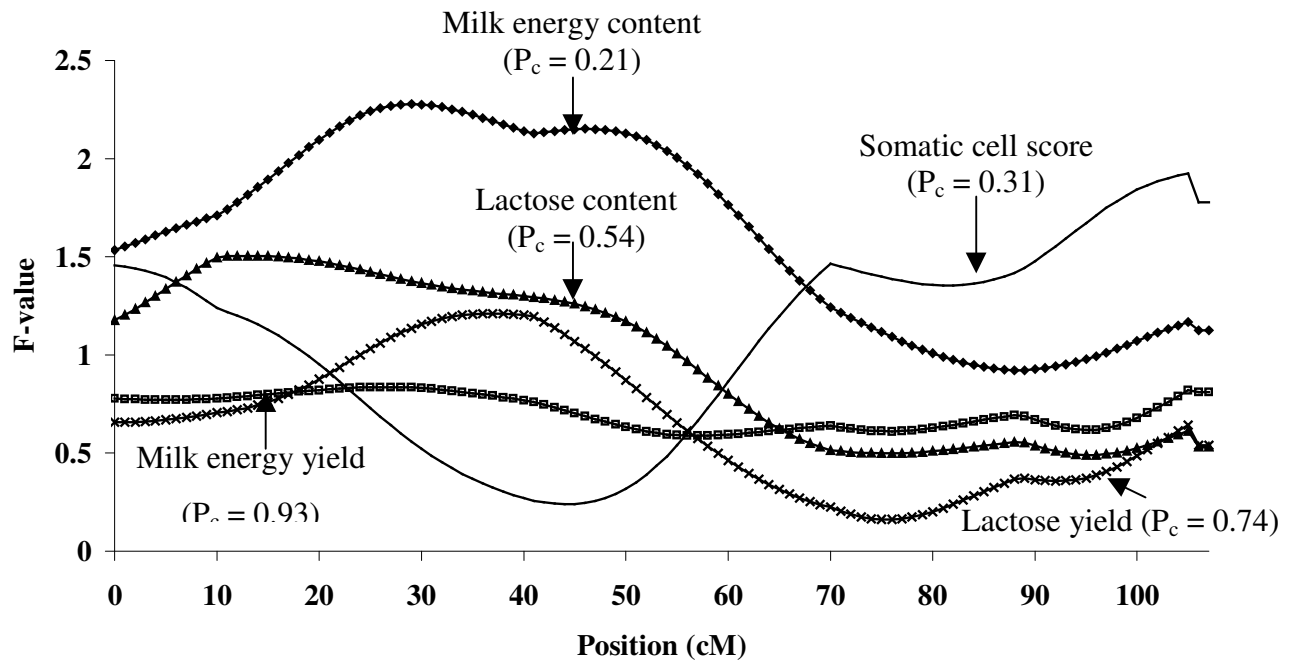


Figure 11: Test statistics for the traits milk energy content and lactose yield as a result of the multimarker regression model included *DGATI K232A*, and the traits milk energy yield, lactose content, and somatic cell score as a result of the classical multimarker regression model (Chapter three) on BTA16.

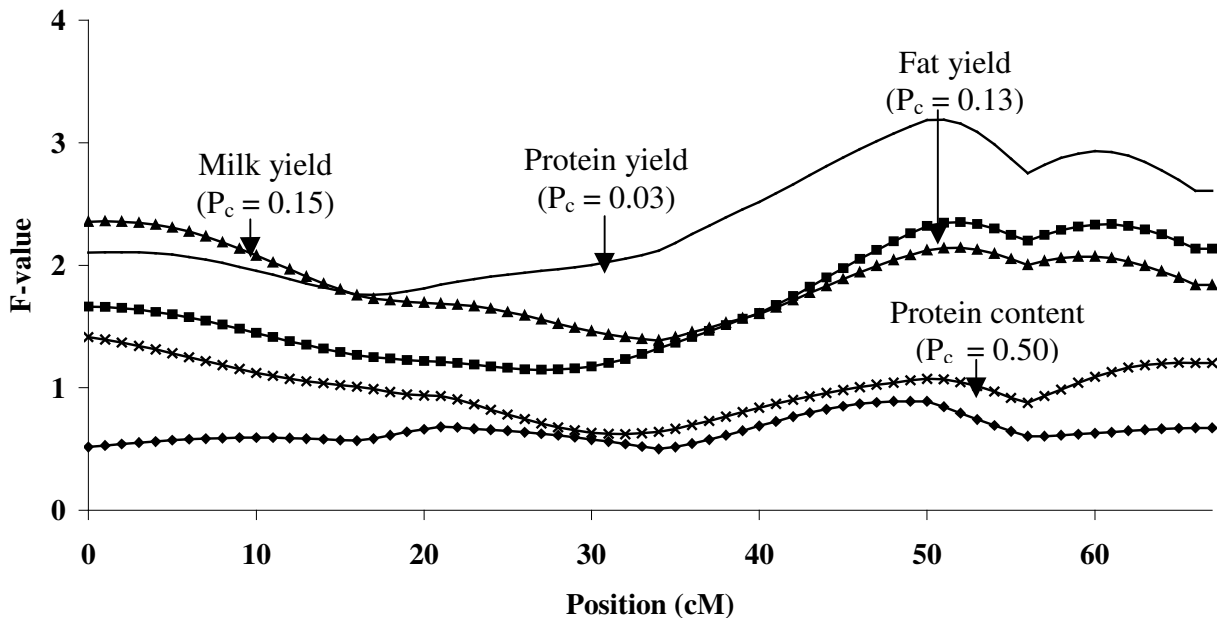


Figure 12: Test statistics for the five traits protein yield and content, fat yield and content, and milk yield on BTA18, results of the multimarker regression model included *DGATI K232A* (Chapter three).

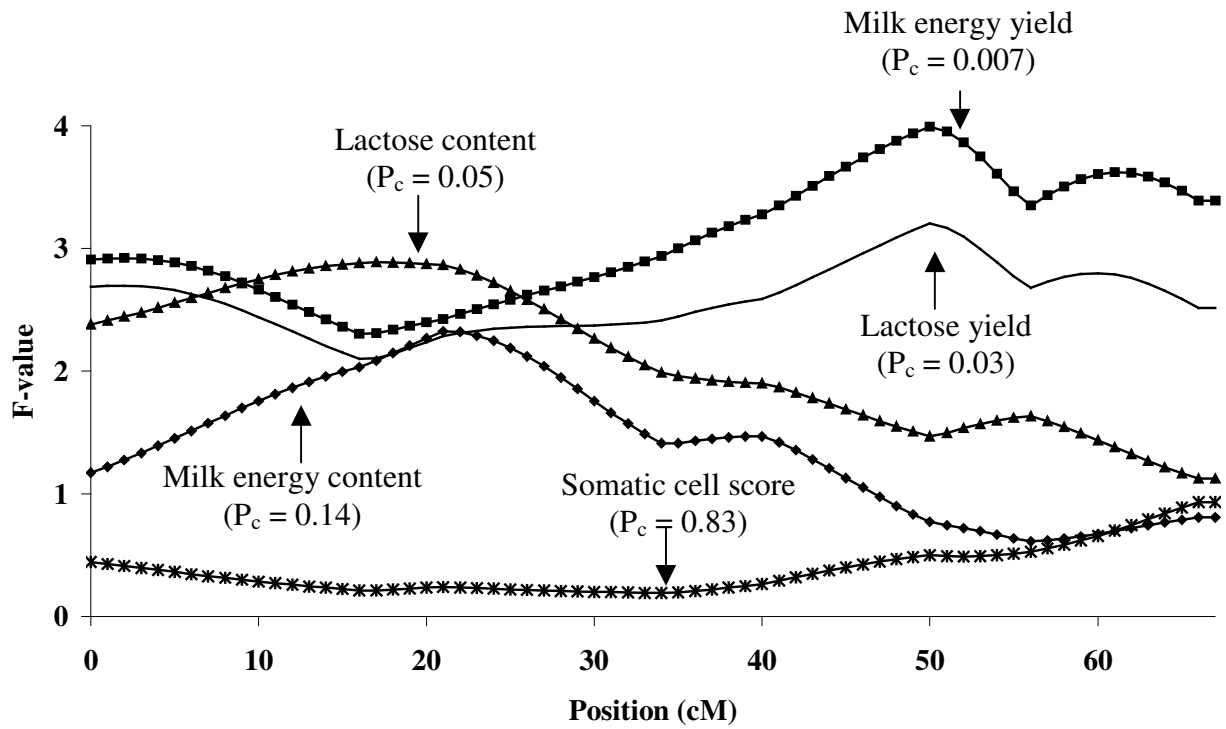


Figure 13: Test statistics for the traits milk energy content and lactose yield as a result of the multimarker regression model included *DGATI K232A*, and the traits milk energy yield, lactose content, and somatic cell score as a result of the classical multimarker regression model (Chapter three) on BTA18.

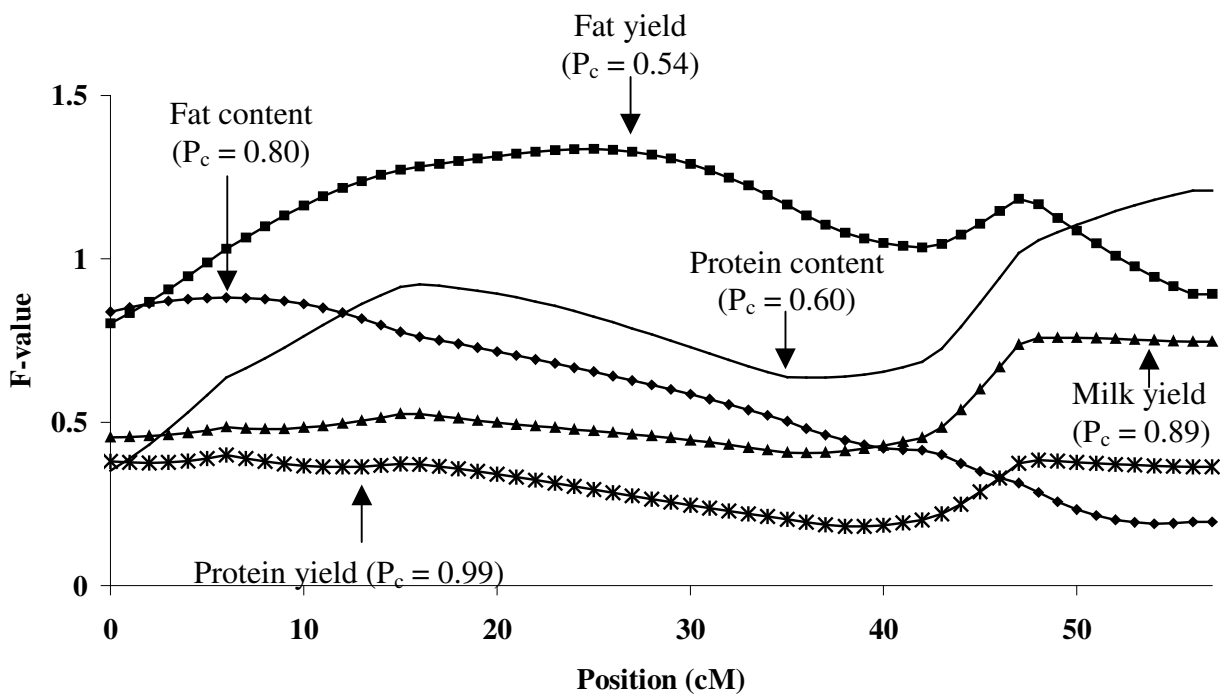


Figure 14: Test statistics for the five traits protein yield and content, fat yield and content, and yield on BTA27, results of the multimarker regression model included *DGATI K232A* (Chapter three).

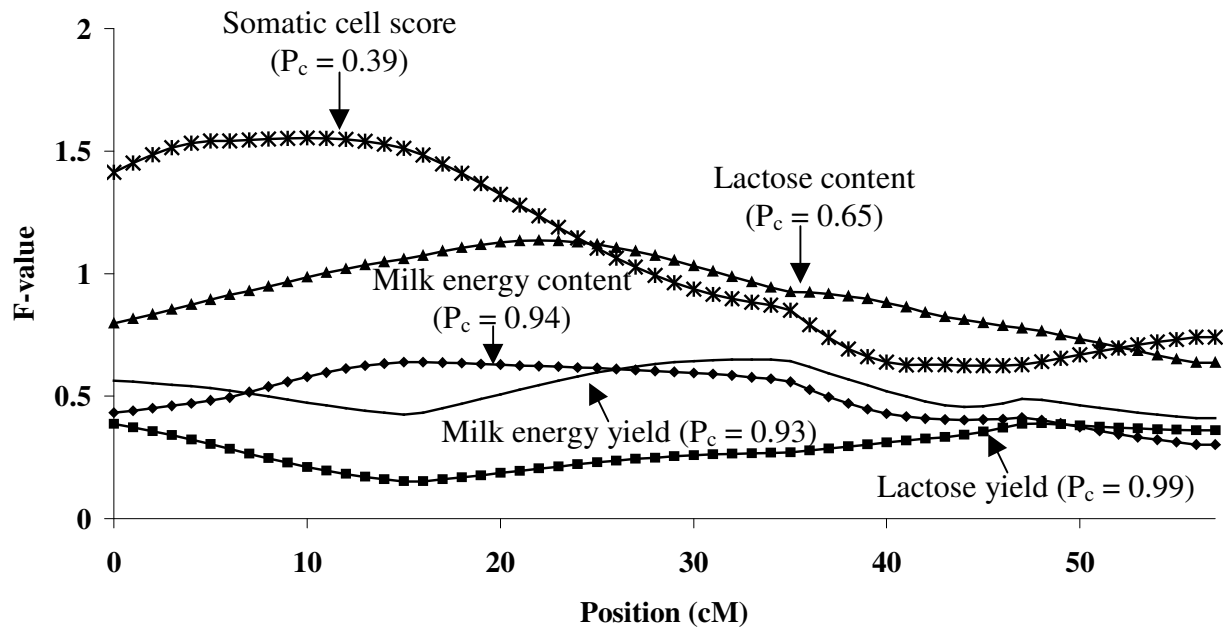


Figure 15: Test statistics for the traits milk energy content and lactose yield as a result of the multimarker regression model included *DGATI K232A*, and the traits milk energy yield, lactose content, and somatic cell score as a result of the classical multimarker regression model (Chapter three) on BTA27.

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