THESIS OF DOCTORAL (PhD) DISSERTATION

GENETIC EVALUATION OF THE LIMOUSIN CATTLE POPULATION IN HUNGARY

WRITTEN BY:

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SZÉCHENYI ISTVÁN UNIVERSITY FACULTY OF
AGRICULTURAL AND FOOD SCIENCES

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1. INTRODUCTION AND OBJECTIVES

Breeding of Limousin cattle is a challenging component of the overall beef business in Hungary. Successful breeding programs can add to the profitability of breeders and well planned programs can also be very rewarding to those developing and implementing them.

Beef cattle breeding, in which the Limousin breed plays a key role, is one of the most dynamically developing sectors of farm animal production in Hungary, despite the fact that beside beef herds, dairy and dual-purpose cattle breeds also play an important role in beef production.

The further development of the beef sector is an important task, both economically and in terms of breeding. Reserves in this area are mainly related to the improvement of reproduction and weaning results of the stocks, the reduction of additional costs, and of feeding procedures.

The reproductive performance of beef cattle herds can be considered favorable if the proportion of weaned calves represents at least 86 per cent of the number of cows in the herd. Under appropriate management conditions all cows give a calf annually, while the calving period does not exceed 90 days.

The main product of the beef cow is the weaned calf. For this reason, improvement of weaning results, namely increasing the weight of weaned calves is an important task, regardless of whether we consider them as breeding animals, as fattening, or as export products.

Population genetic analysis of different traits of the Limousin breed, testing of different breeding value estimation methods in addition to those used so far, their comparison and choosing the most expedient method in this area certainly can add a lot to the profitability of breeders.

As the main product of the domestic export-oriented beef sector are weaned calves sold on foreign markets, population genetic analysis and breeding value estimation, which make up the bulk of present work, are based on the 205-day weaning weight of calves.

It is hoped that results of these studies will contribute to a further development of the weaning weight of Limousin calves.

Application of molecular genetic technologies in the beef industry provide a good opportunity to obtain higher productivity and sustainable animal production. In addition, in case of Limousin herds molecular genetic approaches can contribute to the preservation of the genetic variability. The highly polymorphic microsatellite markers are widely used as genetic markers for purposes that include population genetics, parentage identification, fingerprinting, genetic mapping and conservation. Microsatellite population studies are more frequently applied on local breeds to clarify origin or position of these breeds relative to other ones. Population study can also be performed within a breed to clarify herd position and highlight those populations which require attention by the management. Since studies concerning the genetic diversity and phylogenetic relationship of Limousin cattle on a global scale are few in number and are completely lacking in Hungary, it has been decided to carry out studies within the existing Limousin population with the aim to provide additional data to this particular subject. I also hope that outcome of this study will offer useful data to be utilized in the development of a new breeding strategy of the breed.

In this study, I wished to investigate the following topics:

1. Difference concerning heritability (h^2) of the weaning weight of calves in purebred and crossbred populations by using different animal models for the breeding value estimation
2. Comparison of results obtained by sire and animal models based on real data and interpretation of the variance components, heredity and correlation values.
3. Estimation of the breeding value of Limousin sires with different models (BLUP sire and animal model) in a database of relatives (lateral relatives, offspring) of different genotypes (purebred and crossbred ones). I was curious about the similarity or difference of results obtained by different estimates.

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4. Comparison of purebreeding and crossbreeding breeding value results of the sires, and interpretation of their ranking results with different BLUP models.
5. Comparison of the rank of Limousin sires based on their direct and maternal breeding values. Interpretation of the results regarding the relationship between the two kind of breeding values.
6. Investigation of the phenotypic and genetic trends of weaning results based on the actual, measured values of the 205-day weight and the breeding values estimated by different methods.
7. Since studies concerning the genetic diversity and phylogenetic relationship of Limousin herds are completely lacking in Hungary, I decided to carry out studies in the existing Limousin population with the aim to clarify genetic distance between herds and highlight those which require special attention by the management.

2. MATERIALS AND METHODS

Database of the Association of Hungarian Limousin and Blonde d'Aquitaine Breeders (AHLBB) for weaning results (weaning age, weight and 205-day weigh) were used for breeding value estimation study.

In the first part of this study 13 613 calves (9233 purebred Limousin and 4380 crossbred) were evaluated which came from 37 herds and from 110 different Limousin sires. Considering the genotype of calves, two databases (one of purebred and one of crossbred genotype calves) were formed from the starting data. The resulting three databases were processed with four different BLUP models. During the work, altogether four runnings were performed. Genetic parameters and breeding value of sires were estimated on weaning weight trait.

In the second part of the breeding value estimation study data of 19764 calves (15437 purebred Limousin and 4327 crossbred) were computed. Crossbred calves were sired by Limousin breeding bulls from Simmental dams. Calves in question came from 67 herds and from 338 sires. Direct and maternal breeding value of sires were estimated.

Databases presented above were evaluated with four different BLUP models. Two of them were sire-, and another two were animal models. Both sire models and animal models were run separately on the purebred-, and crossbred calf databases. In the first part of this study direct purebreeding and direct cross-breeding, while in the second part direct and maternal purebreeding and cross-breeding breeding values were estimated.

Sixteen different Limousin cattle herds, maintained for commercial use, were included in the molecular population genetic study. Blood samples were collected for routine parentage testing, by breeders during their established breeding program, from jugular veins from 3,443 individuals (1,520 bulls and 1,924 cows) in tubes containing ethylenediaminetetraacetic acid. Samples were stored at –20°C until

genomic DNA extraction, which was performed using the QIAamp DNA Mini Kit (QIAGEN, Hilden, Germany).

All 18 microsatellite markers used herein (BM1818, BM 1824, BM2113, CSRM60, CSSM66, ETH10, ETH225, ETH3, ILSTS006, INRA23, MGTG4B, RM067, SPS113, SPS115, TGLA 122, TGLA126, TGLA227, TGLA53) are recommended by International Society of Animal Genetics (ISAG) for routine parentage control and record exchange between laboratories

Exact test of Hardy-Weinberg equilibrium (HWE) and exact test of population differentiation were calculated by Genepop 4.2.1, FSTAT 2.9.3.2, and Arlequine programmes. Estimation of exact P value of Hardy-Weinberg test was performed on each locus and each population. Evidence for the presence of null alleles at each locus was evaluated using Genepop and Micro-Checker version 2.2.3 (Monte Carlo simulation; bootstrap method). Observed heterozygosity (H_o), expected heterozygosity (H_e), inbreeding coefficient (F_{IS}), genetic distance (F_{ST}) indices were calculated by Genalex 6.5. Data were crosschecked by FSTAT and Genepop. Bayesian algorithm implemented in Structure was used for inferring the most probable number of clusters (K) and for calculation of membership probability of individuals. For estimation of K, Evanno's method was applied on Structure output. Bayesian stochastic partition-based approach implemented in BAPS 6.0 was also applied to estimate K. A weighted principal component analysis (PCA) was performed using the allele frequency data of Limousin individuals and the 18 microsatellite markers using Genalex. Nei's genetic distance was calculated and viewed by Poptree software. Bootstrap values were based on 1,000 permutations. Assignment tests of individuals were performed by Genalex and Geneclass 2.0 softwares using a Bayesian method and a simulation algorithm with 10,000 simulated individuals.

3. RESULTS AND DISCUSSION

In the four different BLUP models, four different rankings were obtained based on the estimated breeding value of the sires for the weaning weight trait. Results of the breeding value estimation showed that heritability of the weaning weight trait varied within rather wide ranges based on the results of the different runs, but its values ranged from weak to good.

The number of offsprings of Limousin sires, the breeding value of the weaning weight estimated by different BLUP models and the ranking established on the basis of these breeding values based on the **first database,** are presented in Table 1.

Table 1.: Direct breeding value and rank of Limousin sires based on 205-day weaning weight

Registr. number of sires	N		Sire model				Animal model			
			Model 1 (database 1.)		Model 2 (database 2.)		Model 3 (database 1.)		Model 4 (database 2.)	
	Data base 1.	Data-base 2.	Purebred population		Crossbred population		Purebred population		Crossbred population	
			BV	SR	BV	SR	BV	SR	BV	SR
9034	41	298	-0,24	7	-7,53	15	-1,99	6	-1,45	6
11572	298	325	-2,81	12	-9,80	17	-8,56	11	-10,72	10
12015	612	907	-1,90	9	-10,06	18	-8,96	13	-15,84	15
12946	232	259	+1,80	5	-7,36	14	-3,15	7	-11,87	11
13098	917	1483	+1,75	6	-9,09	16	-1,02	4	-10,64	9
13869	244	277	-1,60	8	-6,93	13	-5,93	9	-11,98	12
14284	157	198	-8,16	16	+6,07	9	-15,60	18	-20,78	17
14473	148	201	-8,43	18	+10,22	4	-14,49	17	-13,85	13
14474	184	250	-6,15	15	+9,69	5	-10,77	15	-15,31	14
14476	188	236	-5,91	14	+9,66	6	-12,05	16	-16,78	16
14602	37	187	-8,16	17	-2,77	10	-9,21	14	-22,65	18
14684	721	935	-8,43	19	-3,60	11	-21,15	19	-23,11	19
14712	55	188	+13,79	1	-23,95	20	+25,99	1	+17,29	1
15250	531	687	-14,15	20	-15,13	19	-30,80	20	-39,61	20
16444	436	524	+7,68	2	+9,05	8	+11,94	2	+11,64	4
16496	222	242	-3,13	13	+12,14	3	-7,03	10	-4,15	7
16854	173	239	+3,42	4	+17,65	1	-1,29	5	+13,41	3
17031	150	185	-2,59	11	-5,47	12	-8,59	12	-8,64	8
17562	121	191	+7,40	3	+15,47	2	+4,40	3	+10,97	5
18853	199	203	-1,99	10	+9,30	7	-4,18	8	+15,90	2
M	9233	18746	214,8±5,1		227,4±11,5		214,8±5,1		227,4±11,5	

N = number of offspring of sires; BV = breeding value (kg); SR = rank of sires based on BVE; M= mean value of the population (kg)

Based on our results, it is clear that in case of all sires different breeding values were obtained for the weaning weight trait in the four different BLUP models. For most sires, the direction of breeding values relative to the population average (corrective or degrading effect) was similar, but we found very large differences in numerical values between them (e.g., breeding values of no. 16444 sire in the choice of weight trait: model 1: +7.68 kg model 2: +9.05 kg, model 3: +11.94 kg, model 4: +11.64 kg).

In addition, there were sires in our study (e.g. the no. 18853) whose breeding value had a detrimental effect on the weaning weight trait in purebred populations, but had an improving effect in the crossbred genotype herd. Of the four models, the most prominent results were observed for the sire model (model 2) run on a crossbred genotype herd. Results of the direct and maternal breeding value estimation performed on the **second database** using an animal model are shown in Table 2. Based on the direct and maternal breeding values estimated by the animal model, ranking of the examined Limousin sires showed a larger difference compared to the former study, namely it was opposite, i.e. the ranking of the two breeding values showed a negative correlation coefficient.

Table 2: Direct and maternal breeding value of sires with the most offsprings estimated by 205-day weight using an animal model

| Registr. number of sires	205-day weaning weight (kg)					
	Purebred population			Crossbred population		
	N	Breeding value		N	Breeding vlue	
		Direct	Maternal		Direct	Maternal
9034	61	+2,82	+0,88	394	-5,16	+0,27
12015	608	-7,17	-12,30	257	-18,30	+14,10
12470	43	+7,75	-6,18	50	-4,41	+2,28
12481	88	+1,06	-5,65	66	-14,63	+7,78
12482	54	+11,37	-1,29	54	-16,61	+11,74
12483	79	+7,88	-8,94	56	-20,60	+12,97
12484	72	-1,22	+6,74	59	-10,48	+7,64
12485	49	+9,15	-0,28	60	-13,26	+8,64
13098	1019	-3,17	+3,88	516	+0,13	+0,93
13869	259	-10,88	-4,31	31	+6,38	-5,41
14284	177	-16,77	+4,99	48	+0,08	-0,05
14473	185	-10,27	+2,66	55	+13,07	-10,15
14474	216	-6,51	+4,73	77	-7,31	+4,46
14475	109	-6,39	+2,62	32	+10,09	-6,65
14476	207	-8,09	+4,65	51	-6,58	+4,34
14602	52	-4,13	-1,36	198	-22,89	+13,12
14684	792	-21,92	-3,18	107	-5,73	+3,81
14709	66	-18,34	+10,11	80	+29,46	-19,42
14714	37	-1,43	+0,79	119	+12,85	-8,47
15250	619	-35,39	+22,97	165	-60,03	+39,56
16444	485	9,74	+4,97	62	+12,80	-8,44
16851	80	-10,85	+23,90	36	+7,09	-4,68
16854	202	+1,63	-1,66	45	+33,78	-23,41
17562	153	+3,11	+0,53	52	+23,80	-16,48
21207	86	-8,63	-4,66	31	+21,38	-14,59
Mean value (±SE)	225,7±3,8			221,5±1,23		

N = number of offspring of sires

Results also point out that the direct breeding value and the maternal breeding value of sires are negatively related to each other. Namely, if a breeding bull is selected for mating on its direct breeding value to improve 205-day weight, maternal performance of first-generation heifer will decrease. Notably, their offspring, i.e. the grand-offspring of sires will achieve worse results than the previous generation.

Based on the above-mentioned findings it is worth to consider that breeding values (purebred, crossbred, direct and maternal) of each Limousin sire are different. Therefore, each of them should be estimated and used according to the breeding goal.

Thirty eight of the 288 chi-square tests showed significant deviations from HWE at the 95% confidence interval. Heterozygote excess was calculated in each herd, the highest excess was detected in herd C. Allelic richness ranged between 4.31 and 5.21 (population C and O, respectively). Private alleles were detected in nine herds. Altogether 38 animals (1.1% of the analysed individuals) carried one or more private alleles.

Pairwise exact genotypic differentiation tests performed by FSTAT and Genepop showed that—except the pair B, K—all the herds can be treated as separate units, distinct from each other (p<0.05). Consecutive PCA analysis of estimated F_{ST} values (p<0.000) revealed two herds (C and E) distinct from the majority of other Limousin herds (Figure 1).

Figure 1.: Representation of principal component analysis (PCA) of estimated pairwise FST values obtained by Genalex software.
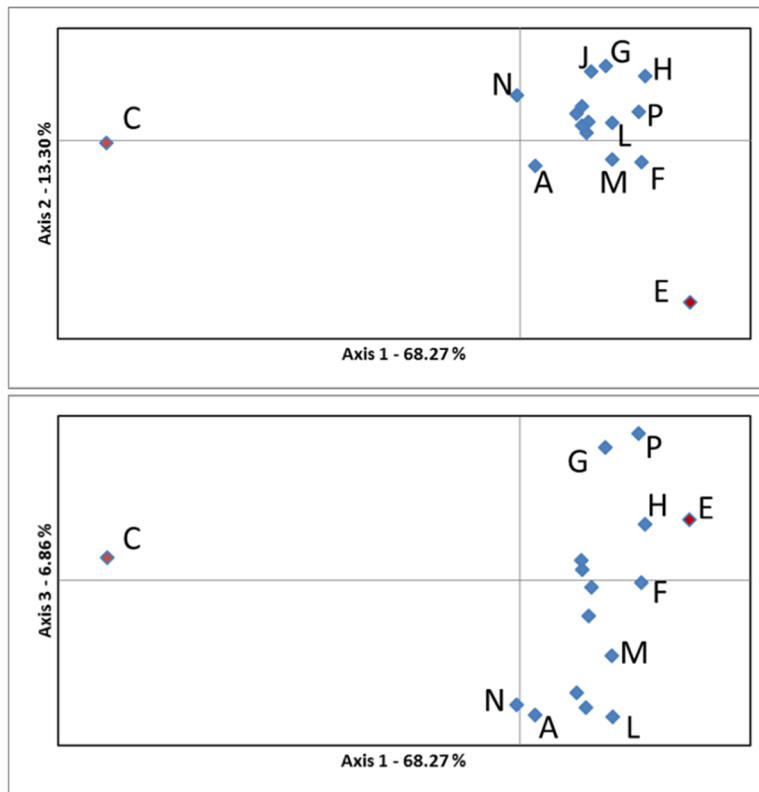
Blue labelled herds are in the range of low genetic differentiation. Red labelled herd C have moderate genetic distance from the other populations, while herd E moderately differentiated from G, H, J, L, N, and P populations. Percentage values represent variation justified by each axis.

UPGMA tree of Nei's genetic distance (Figure 2.) of populations showed the three (A, C, N) herds as distinct groups, but the population E was placed on a distinct branch. This latter observation agrees with the PCA analysis of F_{ST} values. Population B and K remained together on the dendrogram. The remaining groups are the least divergent from each other.

Figure 2.: UPGMA tree of Nei's genetic distance of Limousin cattle from 16 farms. Bootstrap values are indicated on the nodes.
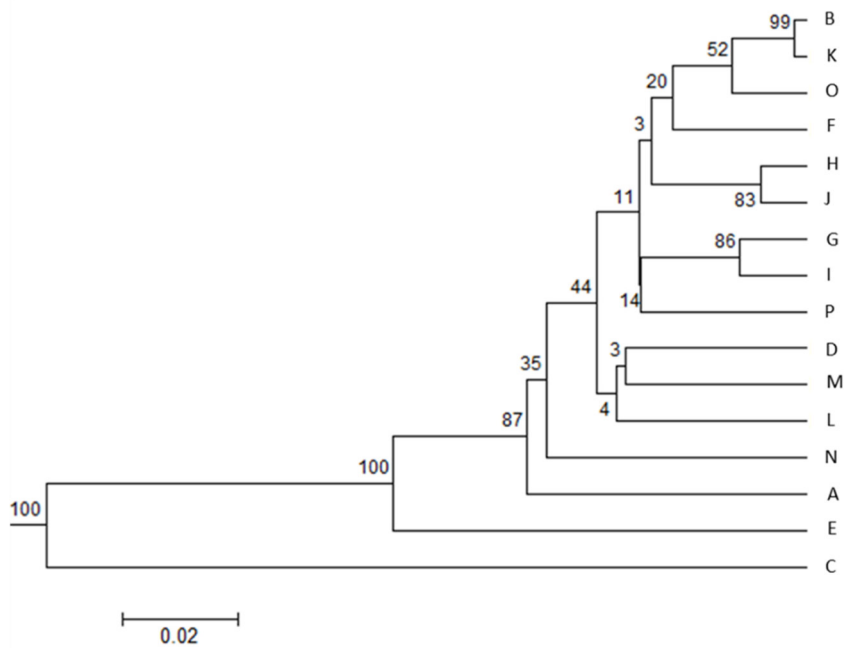
In case of herd A, C, N, and E it is known from the herd books, that cows, semen or embryos have been imported from different regions of France which explain the differences visible on Figure 2. In case of populations C and E the extent of imported individuals were higher than that of the population of A and N.

4. NEW SCIENTIFIC RESULTS

1. Based on sire model there was no difference concerning heritability (h^2) of the weaning weight of calves in purebred and crossbred populations. By using animal model for the breeding value estimation difference between purebred ($h^2=0,28\pm0,05$) and crossbred ($h^2=0,57\pm0,06$) populations was significant.

2. Considerable differences were found in the rank of sires (rrank = 0.32-0.95; $p<0.01$) on purebred, crossbred and mixed databases.

3. Negative correlation was found between the direct and maternal breeding value and that of the rank of the sires based on the two kind of breeding values. Therefore using of sires for mating according to their direct beeding value will result in increased weaning weight of calves in the first generation, followed by a decrease in the second generation.

4. During the last twenty years, there was no genetic progress concerning the 205-day weaning weight of calves in purebred and crossbred Limousin beef cattle populations.

5. Based on F_{st} values C and E herds are moderately differentiated from all other populations.

6. Allelic richness ranged between 4.31 and 5.21 (population C and O, respectively). Altogether 38 animals from 9 herds (D, F, I, J, K, L, N, O és P) carried one or more private alleles.

7. Expected (H_e) and observed (H_o) heterozygosity values in the populations analyzed did not differ significantly, the level of inbreeding (based on the inbreeding coefficient F_{is}) was low.

5. LIST OF PUBLICATIONS

5.1. Publications related to the doctoral dissertation

5.1.1. Paper published in a foreign-language peer-reviewed journals:

M. Szűcs - F. Szabó - B. Bán - Cs. Józsa - L. Rózsa - A. Zsolnai - I. Anton: Assessment of genetic diversity and phylogenetic relationship of Limousin herds in Hungary using microsatellite markers. Asian-Australasian Journal of Animal Science, 2019; 32 (2): 176-182.   IF: 1,227 (Q1)

F. Szabó - **M. Szűcs** - K. Tempfli - D. Berry: Upgrading breeding value estimation in beef cattle. Acta Agraria Debreceniensis, 2018. 150. 451-458.p.

5.1.2. Papers published in a Hungarian-language peer-reviewed journal:

Szabó F. - Balika S. - **Szűcs M.**- Bene Sz. (2007): Limousin borjak választási eredménye. 1. Környezeti hatások (Weaning results of Limousin calves. 1st paper, Environmental effects). Állattenyésztés és Takarmányozás, 56. 6. 541-549.

Bene Sz. - **Szűcs M**. - Polgár J. P. - Szabó F. (2017): Különböző modellekkel becsült örökölhetőségi és tenyészértékek fajtatiszta és keresztezett hústípusú borjak választási adatbázisán (Results of purebred and crossbred beef cattle breeding value estimation based on weaning weight). Állattenyésztés és Takarmányozás, 66. 3. 206-224.

Bene Sz.- **Szűcs M.**- Polgár J.P.- Szabó F. (2017): Fajtán belüli és fajták közötti tenyészértékbecslés a húsmarhatenyésztésben (Within breed

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and across breed breeding value estimation in beef cattle breeding). Állattenyésztés és Takarmányozás, 66. 4. 331-346.

Szűcs M.- Szabó F. - Márton J. - Anton I. -Zsolnai A. - Bene Sz. (2020): Hagyományos tenyészértékbecslési módszerek összehasonlítása limousin húsmarha fajtában (Comparison of different traditional models for breeding value estimation in Limousin beef cattle). Állattenyésztés és takarmányozás **(megjelenés alatt)**.

5.1.3. Conference presentations in Hungarian language

Bene Sz. - **Szűcs M.** - Polgár J. P. - Szabó F. (2017): Tenyészértékek becslése fajtatiszta és vegyes genotípusú húshasznú szarvasmarha populációkban (Breeding value estimation in purebred and crossbred beef cattle populations). LIX. Georgikon Napok, Állattenyésztés és Takarmányozás Szekció. 2017. szeptember 28., Keszthely. 54. ISBN: 978-963-9639-88-1.

Bene Sz. - Polgár J. P. - **Szűcs M.** - Szabó F. (2017): Örökölhetőségi értékek becslése fajtatiszta és vegyes genotípusú húshasznú szarvasmarha populációkban (Estimation of heritability values in purebred and crossbred beef cattle populations). XXIII. ITF, Állattudományok Szekció. 2017. május 26., Keszthely, 1.3.1-6., ISBN: 978-963-9639-87-4.

Szűcs M. – Az értékmérő tulajdonságok alakulása a szarvaltság függvényében a hazai limousin populációban. (Breeding of hornless versus horned Limousin cattle in Hungary) XXXVI. Óvári Tudományos Nap, Mosonmagyaróvár, 2016.november 10. (Szerk.: Bali-Papp Ágnes és Szalka Éva) ISBN 978-615-5391-79-8:283.

5.2. Publications not related to the doctoral dissertation
5.2.1. Papers published in Hungarian-language peer-reviewed journals

Szabó F.- Tempfli K. - Márton I. - Márton J.- **Szűcs M.** - Keller K. (2013): A húsmarhatartás környezetének és genetikai alapjainak bioökonómiai értékelése (Bio-economic evaluation of environment and genetic basis of beef cattle production). Állattenyésztés és Takarmányozás, 62. 4. 398-410.

Szabó F.- Szabó E.- Gulyás L.-Pongrácz L.- Tempfli K. - Kovács Á.- **Szűcs M.**- Keller K. (2014): A legeltetési időszak hosszának hatása a húsmarhák néhány értékmérő tulajdonságának ökonómiai súlyára és a húsmarhatartás eredményességére (The effect of the length of grazing period on the economic weight of some traits of beef cattle and the profitability of cow-calf production). Acta Agronomica Óváriensis. Vol. 56. No 1. 3-12.