

THESES OF DOCTORAL (PhD) DISSERTATION

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**UNIVERSITY OF WEST HUNGARY
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**ROLE OF GENES DETERMINING PRODUCTION TRAITS IN
PUREBRED AND CROSSBRED INDIGENOUS PIG AND CHICKEN
BREEDS**

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1 INTRODUCTION

Recent developments on the sequencing of farm animal genomes provide an increasing amount of information for genotype-trait association studies, population genetic and structure analyses, as well as for the identification of the origin of different livestock species and breeds.

By discovering the molecular genetic background of the associations between genotype and phenotype, faster and more specific improvement can be achieved in animal breeding, along with potential economic benefits. Owing to these advantages, marker-assisted selection is becoming general practice in the breeding of modern, intensive breeds. Results inferred from molecular genetic studies play a more and more important role in breeding value prediction systems and in the construction of commercial lines and populations.

The breakthrough in the applicable methods for genotyping (high resolution melting, fluorescent probes, high-throughput DNA microarrays and DNA-chip technology, new-generation sequencing) provides increasingly wider access to molecular genetic data, promotes the ubiquitous use of genetic information in animal breeding, and means the most promising possibilities for the future improvement of the livestock sector.

Apart from commercial animal production, the significance of molecular genetic markers is increasing also in traditional breeding and maintenance programs of long-established breeds. Molecular markers are now vital tools for the revision of breeding and maintenance schemes.

1.1 Objectives

In purebred Blond Mangalica and crossbred Blond Mangalica×Duroc groups, our objectives were to determine the different genotypes for candidate single nucleotide polymorphisms (SNPs) in melanocortin-4 receptor (*MC4R*) and leptin (*LEP*) genes. *MC4R* and the adipocyte-secreted LEP play pivotal roles in the regulation of fat metabolism and feeding behaviour through hypothalamic areas associated with appetite. Further objectives were to determine the allele and genotype frequencies, and to analyse the effects of the genotypes on several production traits (backfat thickness, loin width, daily gain, ham and shoulder weight, live weight and meat light reflectance value).

In the indigenous Hungarian Yellow chicken population, our aims were to genotype polymorphisms in candidate genes of egg production – such as prolactin (*PRL*) and dopamine receptor D1 (*DRD1*) – and of growth, such as thyroid hormone responsive spot14 α (*Spot14\alpha*), insulin-like growth factor 1 (*IGF1*), insulin-like growth factor-binding protein 2 (*IGFBP2*), and somatostatin (*SST*). Further objectives were to determine allele and genotype frequencies in the population, and to analyse the association between genotypes and important production traits (egg production intensity, egg weight, body weight from hatch to 14 weeks of age, and at 40 and 45 weeks of age).

2 MATERIALS AND METHODS

2.1 Melanocortin-4 receptor (*MC4R*) and leptin (*LEP*) genotyping in crossbred and purebred Blond Mangalica

G1426A *MC4R* and T3469C *LEP* single nucleotide polymorphisms (SNPs) were genotyped in 60 Blond Mangalica (♀) × Duroc (♂) crossbred (F₁) gilts, and in 10 purebred Blond Mangalica barrows. For these animals, slaughter performance and growth-related production data (backfat thickness, ham and shoulder weight, loin width; light reflectance value, live weight, average daily gain) were collected at the abattoir. Further 10 purebred Blond Mangalica boars were also genotyped; however, no production data were accessible for these animals.

Qualification of the pig bodies was carried out by experienced, professional Personnel at the abattoir of Surjányhús Ltd. in Törökszentmiklós. The qualification was implemented in accordance with the relevant, current regulation (136/2011. (XII. 22.) VM decree, 2011). Live weight, ham and shoulder weight were measured using certified scales at the slaughterhouse. Fat-o-Meater system was used to measure backfat thickness, loin width and light reflectance.

The animals involved were raised under identical housing and feeding conditions at the farm of the OLMOS & TÓTH Ltd. located in Nyíribrony.

For DNA extraction, hair follicle samples were collected and Wizard Genomic DNA Purification kit (Promega, USA) was used. Genotyping was carried out by the means of polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method.

2.2 Genotype studies in Hungarian Yellow hens

436 animals were genotyped for the 24-base-pair indel in prolactin (*PRL*), the G123A SNP in dopamine receptor D1 (*DRD1*), and the A213C SNP in thyroid hormone responsive spot14alpha (*Spot14a*) genes, respectively. Promising SNPs in insulin-like growth factor 1 (*IGF1*), insulin-like growth factor-binding protein 2 (*IGFBP2*), and somatostatin (*SST*) genes were also analyzed in 110 hens.

The hens were hatched and raised at the genetic resource farm located in Mosonmagyaróvár, managed by the University of West Hungary. The experimental population was kept under the same feeding and housing conditions with *ad libitum* access to food and water. For genotype-trait associations, body weight measurements were taken biweekly from the day of hatching to 14 weeks of age, and egg production was monitored between 40 and 45 weeks of age. Body weight was also recorded at the beginning and the end of the egg collection period. Eggs laid during this period were measured and an average egg weight was determined. Egg production intensity (EPI) was calculated as follows: $EPI = (\text{number of eggs laid/days of egg collection period}) \times 100$. Blood samples were drawn from wing veins into collection tubes (SARSTEDT, Germany) supplied with EDTA as anticoagulant. DNA extraction from whole blood was carried out by means of the Wizard Genomic DNA Isolation Kit (Promega, USA). PCR-RFLP was used for genotyping. 4-4 PCR products of each locus were sequenced to verify the amplified regions and to uncover possible new variations. Sequencing was carried out with either 3730xl DNA Analyzer or PRISM 3100 Genetic Analyzer (Applied Biosystems, USA).

2.3 Statistical analyses

Excel (Microsoft, 2003, USA) software was used for data collection and management.

Normality of data distribution was analysed by Kolgomorov–Smirnov tests in SPSS for Windows v.16.0 (SPSS, USA) statistic software package. Genotype-trait associations in pigs were evaluated by LSD (least significant difference) and Mann–Whitney tests.

HARDY–WEINBERG equilibrium was analysed by chi-square (χ^2) tests of the observed and expected genotype frequencies both in the pig groups and in the Hungarian Yellow chicken population.

The effects of *PRL*, *DRD1* and *Spot14a* genotypes on production traits were analysed with general linear method (GLM procedure) using the following model:

$$Y = \mu + H + P + G_{PRL} + G_{DRD1} + G_{Spot14a} + e,$$

where: Y = phenotypic records of the analysed traits (body weight at different ages, egg weight, egg production intensity); μ = population mean of the traits; H = effect of hatching; P = effect of the floor-pen; G_{PRL} = effect of the *PRL* genotype; G_{DRD1} = effect of the *DRD1* genotype; $G_{Spot14a}$ = effect of the *Spot14a* genotype; e = residual random error.

Genotype interactions ($G_{PRL} \times G_{DRD1}$; $G_{PRL} \times G_{Spot14a}$; $G_{DRD1} \times G_{Spot14a}$) were also tested but not significant ($P > 0.05$).

3 RESULTS

3.1 Results in the crossbred and purebred Mangalica groups

3.1.1 *MC4R* genotype

The G1426A polymorphism was present in both crossbred and purebred Mangalica. Three genotypes (*GG*, *AG* and *AA*) were separated in 60 Blond Mangalica×Duroc individuals, whereas two genotypes (*GG*, and *AG*) were observed in 20 purebred Blond Mangalica pigs. The following restriction fragments were identified: 156 and 70 bp for genotype *GG*, 226, 156 and 70 bp for genotype *AG*, and 226 bp for genotype *AA*. Allele and genotype frequencies for the different groups are presented in **Table 1** and **2**.

Table 1. *MC4R* allele and genotype frequency (%) in the crossbred (F₁) group, chi-square test results for HARDY–WEINBERG equilibrium (degree of freedom (df) = 2).

Allele frequency	Genotype frequency (n)*	χ^2	P
<i>G</i> = 73	<i>GG</i> (30) = 50 / 52.5	0.99	0.61
<i>A</i> = 27	<i>AG</i> (27) = 45 / 40		
	<i>AA</i> (3) = 5 / 7.5		

* The observed and the HWE expected genotype frequencies are presented (%) and separated by a slash

Table 2. *MC4R* allele and genotype frequency (%) in the purebred group, chi-square test results for HARDY–WEINBERG equilibrium (degree of freedom (df) = 2).

Allele frequency	Genotype frequency (n)*	χ^2	P
<i>G</i> = 78	<i>GG</i> (11) = 55 / 60	1.69	0.37
<i>A</i> = 22	<i>AG</i> (9) = 45 / 35		
	<i>AA</i> (0) = 0 / 5		

* The observed and the HWE expected genotype frequencies are presented (%) and separated by a slash

In the crossbred group, *MC4R* genotype was significantly ($P < 0.05$) associated with backfat thickness at both measuring points. These results confirm the contribution of allele *A* to a more intensive fat deposition. Further significant ($P < 0.05$) association was uncovered between *MC4R* genotype and the Fat-o-Meater light reflectance values of meat (**Table 3**). The loin of homozygous *G* animals was characterized by the lowest light reflectance values (indicating meat darker in colour), as compared to the heterozygotes and the homozygous *A* individuals. None of the other analysed traits were significantly ($P > 0.05$) influenced by *MC4R* genotype; however, a positive effect of allele *A* was observed on average daily gain, ham and shoulder weight.

Table 3. G1426A *MC4R* genotype effects on different traits (mean \pm standard deviation) in the crossbred (F_1) group.

Traits	<i>MC4R</i> genotype (n)		
	<i>GG</i> (30)	<i>AG</i> (27)	<i>AA</i> (3)
Backfat 1 (mm)	41.4 \pm 4.7^a	45.1 \pm 3.8^b	47.0 \pm 2.6^b
Backfat 2 (mm)	38.0 \pm 4.8^a	41.4 \pm 5.6^b	47.7 \pm 3.8^c
Average daily gain (g)	701.7 \pm 54.1	715.2 \pm 60.7	753.3 \pm 32.1
Live weight (kg)	140.5 \pm 6.93	140.0 \pm 8.1	147.3 \pm 4.6
Age (day)	239.4 \pm 15.7	237.4 \pm 13.1	238.3 \pm 7.1
Right ham (kg)	11.63 \pm 0.77	11.60 \pm 0.89	12.10 \pm 0.81
Left ham (kg)	11.66 \pm 0.74	11.60 \pm 0.82	11.84 \pm 0.60
Right shoulder (kg)	7.20 \pm 0.40	7.10 \pm 0.49	7.40 \pm 0.75
Left shoulder (kg)	7.16 \pm 0.42	7.25 \pm 0.54	7.49 \pm 0.94
Loin width (mm)	55.1 \pm 5.2	53.6 \pm 6.8	50.5 \pm 12.0
Light reflectance	34.7 \pm 2.8^a	36.9 \pm 5.0^b	42.3 \pm 4.7^c
Arrival weight (kg)	34.8 \pm 5.8	35.7 \pm 6.6	33.6 \pm 3.7
Fattening period (day)	148.5 \pm 7.6	146.7 \pm 9.3	151.0 \pm 0.0

^{a,b,c} Values with different superscripts in the same row differ significantly ($P < 0.05$)

3.1.2 *LEP* genotype

Only *TT* and *TC* genotypes were detected in both the Blond Mangalica × Duroc F₁ and the purebred Blond Mangalica groups, which demonstrates the low prevalence of the *C* allele (**Table 4**).

The following fragments were separated after the digestion with *HinfI* restriction enzyme: 397 and 89 bp for *TT* genotype, 397, 347, 89 and 50 bp for *TC* genotype.

Table 4. *LEP* allele and genotype frequencies (%) in crossbred (F₁) and purebred animals.

Group (n)	Allele frequency	Genotype frequency
F ₁ (60)	<i>T</i> = 93	<i>TT</i> = 87
	<i>C</i> = 7	<i>TC</i> = 13
		<i>CC</i> = 0
Mangalica (20)	<i>T</i> = 80	<i>TT</i> = 60
	<i>C</i> = 20	<i>TC</i> = 40
		<i>CC</i> = 0

LEP genotype was associated ($P < 0.05$) with average daily gain in the crossbred (F₁) group (**Table 5**). Allele *C* was beneficial for this trait: heterozygous animals surpassed the homozygotes by more than 50 g daily gain during the fattening period. Other traits were not significantly ($P > 0.05$) influenced by the *LEP* genotype.

The synonymous T3469C *LEP* polymorphism can affect production traits by the modification of transcript stability and translational efficiency, or can be closely linked to causative non-synonymous mutations.

Table 5. T3469C *LEP* genotype effects on different traits (mean \pm standard deviation) in the crossbred (F_1) population.

Traits	<i>LEP</i> genotype (n)	
	<i>TT</i> (52)	<i>TC</i> (8)
Backfat 1 (mm)	43.2 \pm 4.6	43.9 \pm 5.2
Backfat 2 (mm)	39.9 \pm 5.8	40.4 \pm 4.8
Average daily gain (g)	703.3 \pm 51.5^a	756.3 \pm 71.7^b
Live weight (kg)	140.62 \pm 7.57	140.63 \pm 7.11
Age (day)	239.4 \pm 13.3	232.6 \pm 18.5
Right ham (kg)	11.65 \pm 0.82	11.62 \pm 0.93
Left ham (kg)	11.67 \pm 0.75	11.43 \pm 0.85
Right shoulder (kg)	7.19 \pm 0.45	7.05 \pm 0.53
Left shoulder (kg)	7.24 \pm 0.47	7.07 \pm 0.65
Loin width (mm)	54.9 \pm 5.1	49.2 \pm 11.1
Light reflectance value	36.3 \pm 4.1	34.6 \pm 6.0
Arrival weight (kg)	35.6 \pm 6.1	31.7 \pm 5.1
Fattening period (day)	148.2 \pm 7.5	144.6 \pm 11.9

^{a,b} Values with different superscripts in the same row differ significantly ($P < 0.05$)

3.2 Results in Hungarian Yellow hens

3.2.1 *PRL* genotype

The analysed *PRL* indel was polymorphic in the population, two alleles and three genotypes (*DD*, *ID*, *II*) were detected. The *I* allele was represented by a 201 bp PCR-product, whereas the *D* allele was 177 bp long. Allele and genotype frequencies are presented in **Table 6**.

From chi-square test results, it can be inferred that the genotypes distribute according to HWE (**Table 6**). The high heterozygosity may be attributed to the breeding program that supports the mating of nonrelated individuals by mixing the males from one floor-pen with the females of the adjacent pen from year to year.

Table 6. *PRL* allele and genotype frequencies (%), results of the chi-square test for HARDY–WEINBERG equilibrium, polymorphism information content (PIC) and heterozygosity (He) in the Hungarian Yellow population.

Allele frequency	Genotype frequency ^a	χ^2	P-value ^b	PIC	He
<i>I</i> = 53 <i>D</i> = 47	<i>DD</i> = 23 (22) <i>ID</i> = 48 (50) <i>II</i> = 29 (28)	0.511	0.47	0.37	0.50

^a HWE expected frequencies are presented in parentheses

^b Degree of freedom (df) = 1

PRL genotype was associated ($P < 0.05$) with egg production intensity, and had no significant ($P > 0.05$) effect on body or egg weight in the monitoring period (**Table 7**). The insertion allele proved to be more beneficial for egg production, which is in agreement with the allele substitution pattern in several other breeds and crosses.

Table 7. *PRL* genotype effects on various traits (estimated marginal mean \pm standard error).

Traits	<i>PRL</i> genotype (n)		
	<i>DD</i> (102)	<i>ID</i> (210)	<i>II</i> (124)
Body weight at week 8 (g)	541.8 \pm 5.8	560.4 \pm 3.7	568.2 \pm 5.9
Body weight at week 10 (g)	789.4 \pm 7.9	806.2 \pm 6.3	793.3 \pm 5.9
Body weight at week 12 (g)	886.7 \pm 10.5	915.8 \pm 6.8	913.4 \pm 7.7
Body weight at week 14 (g)	969.6 \pm 16.4	1003.4 \pm 12.7	1010.8 \pm 14.0
Body weight at week 40 (g)	1757.1 \pm 18.4	1791.7 \pm 17.3	1772.7 \pm 16.6
Body weight at week 45 (g)	1941.2 \pm 21.94	1961.7 \pm 15.39	1911.3 \pm 22.19
Egg weight (g)	54.85 \pm 0.47	52.83 \pm 0.49	53.78 \pm 0.35
Egg production intensity (pcs)	49.52 \pm 1.11^b	55.76 \pm 0.83^a	55.08 \pm 0.81^a

^{a, b} Values with different superscripts in the same row differ significantly ($P < 0.05$)

3.2.2 *DRD1* genotype

After *BsrSI* enzyme digestion, the following three genotypes were separated in the Hungarian Yellow population: *GG* (283 bp), *AG* (111, 172, 283 bp fragments), and *AA* (111, 172 bp fragments). Allele and genotype frequencies are presented in **Table 8**. Non-significant ($P > 0.05$) deviation of the observed from the expected frequencies was detected, indicating HWE in the population.

Table 8. *DRD1* G123A allele and genotype frequencies (%), results of the chi-square test for HARDY–WEINBERG equilibrium, polymorphism information content (PIC) and heterozygosity (He) in the Hungarian Yellow population.

Allele frequency	Genotype frequency ^a	χ^2	P-value ^b	PIC	He
<i>G</i> = 58	<i>AA</i> = 15 (17)	3.001	0.08	0.37	0.49
<i>A</i> = 42	<i>AG</i> = 53 (49)				
	<i>GG</i> = 32 (34)				

^a HWE expected frequencies are presented in parentheses

^b Degree of freedom (df) = 1

DRD1 genotype affected egg production intensity and body weight of mature individuals at 45 weeks of age (**Table 9**). Allele *A* can be considered advantageous in Hungarian Yellow chicken. However, the analyzed G123A is a silent (Thr/Thr) mutation in the transmembrane domain of *DRD1*, synonymous variations can also affect the phenotype through modification of splicing and RNA stability, or may be closely linked to the causative loci.

Genotype and body weight associations have not yet been reported for the SNP; nevertheless, D1 receptor and its agonists' levels have been shown to influence eating behaviour and body weight in humans and rodents.

Table 9. *DRD1* genotype effects on various traits (estimated marginal mean \pm standard error).

Traits	<i>DRD1</i> genotype (n)		
	AA (68)	AG (230)	GG (138)
Body weight at week 8 (g)	559.6 \pm 6.1	559.3 \pm 3.2	556.0 \pm 4.9
Body weight at week 10 (g)	808.8 \pm 9.9	799.1 \pm 5.2	792.8 \pm 5.6
Body weight at week 12 (g)	902.1 \pm 9.9	916.0 \pm 7.9	898.5 \pm 8.0
Body weight at week 14 (g)	1006.9 \pm 17.7	1001.3 \pm 12.3	986.8 \pm 13.9
Body weight at week 40 (g)	1788.1 \pm 21.8	1781.9 \pm 16.1	1767.2 \pm 17.2
Body weight at week 45 (g)	1995.6 \pm 26.5^a	1944.9 \pm 14.5^{ab}	1913.0 \pm 21.1^b
Egg weight (g)	52.81 \pm 0.98	53.47 \pm 0.29	54.11 \pm 0.35
Egg production intensity (pcs)	59.92 \pm 1.14^a	53.89 \pm 0.72^b	51.61 \pm 0.78^b

^{a, b, c} Values with different superscripts in the same row differ significantly (P<0.05)

4.2.3 *Spot14a* genotype

After *Bsa*HI enzyme digestion, the following genotypes were discriminated: AA (419 bp), AC (419, 319, 100 bp fragments), CC (319, 100 bp).

Table 10. *Spot14a* A213C allele and genotype frequencies (%), results of the chi-square test for HARDY–WEINBERG equilibrium, polymorphism information content (PIC) and heterozygosity (He) in the Hungarian Yellow population.

Allele frequency	Genotype frequency ^a	χ^2	P-value ^b	PIC	He
C = 62	AA = 13 (15)	2.213	0.14	0.36	0.47
A = 38	AC = 51 (47)				
	CC = 36 (38)				

^a HWE expected frequencies are presented in parentheses

^b Degree of freedom (df) = 1

Spot14a genotype influenced body weight from 8 to 14, at 40 and 45 weeks of age, and egg weight between 40 and 45 weeks of age (**Table 11**). Genotype effects are stronger ($P < 0.01$) at older ages, and at greater body weight. No significant genotype associations were detected in Hungarian Yellow with hatch weight, and body weight from 2 to 6 weeks of age.

The *Spot14a* A213C SNP is non-synonymous and results in aspartic (allele C) to glutamic (allele A) acid change. In the Hungarian Yellow population, the A allele was found more favourable, and indicated an increase in body weight.

Based on our results and literature data, a non-direct effect of the SNP can be suggested, where the actual causative mutation is closely linked to A213C, and is in inverse linkage in different populations. Before adopting this potential *Spot14a* SNP to marker-assisted selection programs, special attention should be paid to survey the actual direction of the allele substitution effect in the given breed or line. Previously, *Spot14a* SNPs have not been associated with egg weight in other breeds or lines.

Table 11. *Spot14a* genotype effects on various traits (estimated marginal mean \pm standard error).

Traits	<i>Spot14a</i> genotype (n)		
	AA (57)	AC (221)	CC (158)
Body weight at week 8 (g)	588.1 \pm 7.9 ^a	555.1 \pm 3.3 ^b	552.0 \pm 4.2 ^b
Body weight at week 10 (g)	825.2 \pm 9.3 ^a	799.2 \pm 5.4 ^b	788.2 \pm 6.5 ^b
Body weight at week 12(g)	972.1 \pm 11.8 ^a	912.3 \pm 7.2 ^b	879.7 \pm 6.9 ^c
Body weight at week 14 (g)	1091.9 \pm 19.7 ^a	999.3 \pm 12.4 ^b	961.2 \pm 14.2 ^c
Body weight at week 40 (g)	1899.1 \pm 23.8 ^A	1793.7 \pm 14.9 ^B	1712.9 \pm 18.4 ^C
Body weight at week 45 (g)	2072.2 \pm 29.7 ^A	1949.8 \pm 15.9 ^B	1886.1 \pm 20.3 ^C
Egg weight (g)	56.98 \pm 0.97 ^a	52.68 \pm 0.35 ^b	53.59 \pm 0.36 ^b
Egg production intensity (pcs)	53.76 \pm 1.18	53.47 \pm 0.72	55.13 \pm 0.80

^{a, b, c} Values with different superscripts in the same row differ significantly ($P < 0.05$)

^{A, B, C} Values with different superscripts in the same row differ significantly ($P < 0.01$)

4.2.4 *IGF1*, *IGFBP2* and *SST* genotype

The *C* allele at the A570C locus in *IGF1*, the *G* allele at the G645T locus in *IGFBP2*, and the *A* allele at the A370G locus in *SST* seem to be fixed in the Hungarian Yellow population. *IGF1 C* allele was represented by 191 and 622 bp fragments. *IGFBP2 G* allele also had two diagnostic fragments (117 and 198 bp), whereas *SST A* allele was identified by the uncut 330 bp long product.

4.2.5 Sequencing results

PCR-product sequences of the six analyzed genes were aligned, and were also run against the chicken nucleotide database in Basic Local Alignment Search Tool (BLAST). Sequencing and BLAST search retrieved several polymorphisms previously described in other breeds: 1) four SNPs in the promoter of *PRL*, of which three *T-C* transitions at the positions 105 (allele *T* was present in the Yellow Hungarian hen samples), 129 (allele *T*), 176 (allele *T*), and one A151G (allele *A*) transition (positions according to GenBank accession number FJ434669); 2) a synonymous (Ala/Ala) T201C transition (allele *T*) in the coding region of *DRD1* (GenBank NM_001144848); 3) one synonymous (Ala/Ala) G137A transition (allele *G*), and a 9 bp indel (from base 255 to 263; the deletion was present in Hungarian Yellow) in *Spot14a* coding region (GenBank AY568628). A novel (not reported in GenBank) non-synonymous (Arg/Lys) A166G SNP occurred in *Spot14a* (position in GenBank AY568628), and another novel synonymous (Pro/Pro) T1226C transition was detected in *SST* (GenBank AY555066).

5 NEW SCIENTIFIC RESULTS

1. G1426A *MC4R* and T3469C *LEP* genotypes were determined in purebred Blond Mangalica and in crossbred Blonde Mangalica×Duroc (F₁) groups. Two *MC4R* genotypes were observed in the purebred group, whereas three genotypes were present in the crossbred population. In both groups, only two genotypes (*TT*, *TC*) were observed for the *LEP* polymorphism, which implies the low frequency of the *C* allele.

2. In the crossbred group, *MC4R* and *LEP* genotype association studies were carried out for traits related to growth and meat production. Significant ($P<0.05$) associations were observed between the *MC4R* genotype and backfat thickness, and light reflectance values. *LEP* genotype significantly ($P<0.05$) affected average daily gain. The crossbred group significantly ($P<0.05$) surpassed the purebred animals regarding most of the monitored traits.

3. The *C* allele at the A570C locus in *IGF1*, the *G* allele at the G645T locus in *IGFBP2*, and the *A* allele at the A370G locus in *SST* are fixed in the Hungarian Yellow chicken population. 3-3 genotypes were present for *PRL*, *DRD1*, and *Spot14α* polymorphisms, respectively. These genotypes distribute according to HWE.

4. The *PRL* indel genotype was associated ($P < 0.05$) with egg production intensity, and had no significant ($P > 0.05$) effect on body or egg weight in the monitoring period. The *I* allele proved to be more beneficial for egg production in the Hungarian Yellow hens.

5. *DRD1* genotype affected egg production and body weight of mature individuals at 45 weeks of age. Allele *A* is considered advantageous in Hungarian Yellow chicken.

6. *Spot14a* genotype influenced body weight from 8 to 14, at 40 and 45 weeks of age, and egg weight between 40 and 45 weeks of age. Genotype effects are stronger ($P < 0.01$) at older ages and greater body weight. No significant genotype associations were detected in Hungarian Yellow with hatch weight, and body weight from 2 to 6 weeks of age. In the Hungarian Yellow population, the *A* allele is more favourable.

7. Through sequencing and BLAST search, a novel (not reported in GenBank) non-synonymous polymorphism was detected in *Spot14a*, and another novel synonymous SNP was identified in *SST*.

5 List of publications

5.1 Scientific papers published in peer-reviewed journals (in Hungarian)

1. TEMPFLI K. – SIMON Zs. – SIMON Z. – BALI PAPP Á. (2013): A melanokortin-4 receptor és a leptin polimorfizmusának vizsgálata mangalica×duroc és mangalica sertésekben. Magyar Állatorvosok Lapja, 135(6). 339–344. (IF: 0.146*)
2. TEMPFLI K. – TÓTH P. – SIMON Z. – SZÜCS E. – BALI PAPP Á. (2012): A sertéshús telítetlen zsírsavtartalmának növelése és hatásai a zsírsanyagcsere genetikai szabályozására. Magyar Állatorvosok Lapja, 134(3). 150–156. (IF: 0.146)
3. TEMPFLI K. – GAJDÓCSI E. – BALI PAPP Á. (2010): A gének szerepe a sertéshús minőségének alakításában. Magyar Állatorvosok Lapja, 132(5). 259–264. (IF: 0.3)

5.2 Scientific papers published / under review in peer-reviewed journals (in English)

1. TEMPFLI, K. – KONRÁD, SZ. – KOVÁCSNÉ GAÁL, K. – PONGRÁCZ, L. – BALI PAPP, Á.: Prolactin, dopamine receptor D1 and Spot14a polymorphisms affect production traits of Hungarian Yellow hens. Livestock Science, under review
2. TEMPFLI, K. – FARKAS, G. – SIMON, Zs. – BALI PAPP, Á. (2011): Effects of prolactin receptor genotype on the litter size of Mangalica. Acta Veterinaria Hungarica, 59(2). 269–277. (IF: 0.673)

5.3 Conference presentation (in Hungarian)

1. TEMPFLI K. – KONRÁD SZ. – KOVÁCSNÉ GAÁL K. – BALI PAPP Á. (2012): Prolaktin polimorfizmus hatása a tojástermelési tulajdonságokra sárga magyar tyúkoknál. XXXIV Óvári Tudományos Nap, Mosonmagyaróvár, október 5. (oral presentation)

5.4 Conference presentations (in English)

1. TEMPFLI, K. – KONRÁD, SZ. – KOVÁCSNÉ GAÁL, K. – BALI PAPP, Á. (2012): PRLR and PRL polymorphism studies in Mangalica pig and Hungarian Yellow chicken. The Impact of Urbanization, Industrial, Agricultural and Forest Technologies on the Natural Environment – International Scientific Conference on Sustainable Development & Ecological Footprint, Sopron, March 27. p.1-6. (oral presentation)
2. TEMPFLI, K. – BALI PAPP, Á. (2011): Evaluation of the effects of prolactin receptor genotype on the litter size of Mangalica. Fatty Pig – Science and Utilization International Conference, Herceghalom, November 18. p.25. (oral presentation)

5.5 Conference proceedings

1. TEMPFLI, K. – BALI PAPP, Á. (2011): Polymorphism of prolactin receptor gene and effect on the litter size of native Hungarian pig breed. Plant & Animal Genome Conference XIX, San Diego, USA, január 14-19. p.237. (poster presentation)
2. TEMPFLI, K. – SIMON ZS. – BALI PAPP, Á. (2010): A prolaktin receptor gén alléljainak hatása a mangalica alomméretére. XXXIII Óvári Tudományos Nap, Mosonmagyaróvár, október 7. (poster presentation)
3. GAJDÓCSI, E. – PATAKI, R. – VARGA, E. – KISS, R. – TEMPFLI, K. – BALI PAPP, Á. (2008): The effect of the gene of prolactin receptor on Mangalica pigs' litter size. XXXII Óvári Tudományos Nap, Mosonmagyaróvár, október 9. (poster presentation)
4. GAJDÓCSI E. – PATAKI R. – TEMPFLI, K. – BALI PAPP, Á. (2008): A prolaktin receptor gén hatása a mangalicák alomméretére. I Gödöllői Állattenyésztési Tudományos Napok. Gödöllő, április 11-12. (poster presentation)

5.6 Scientific papers not related to the dissertation (in Hungarian)

1. SZABÓ F. – TEMPFLI K. – MÁRTON I. – MÁRTON J. – SZÜCS M. – KELLER K. (2013): A húsmarhatartás környezetének és genetikai alapjainak bio-ökonómiai értékelése. *Állattenyésztés és Takarmányozás*, 62(4). 398–410.
2. VARGA E. – EGRSZEGI I. – RÁTKY J. – KISS R. – TEMPFLI K. – BALI PAPP Á. (2009): A zona pellucidában vitrifikáció után bekövetkezett változások összehasonlítása *in vivo* és *in vitro* érlelt csupasz és kumulusz sejtés sertés petesejteknel. *Acta Agronomica Óváriensis*, 51. 39–50.
3. VARGA E. – EGRSZEGI I. – RÁTKY J. – TEMPFLI K. – PATAKI R. – BALI PAPP Á. (2009): Mangalica petesejtek és embriók krioprezervációja. *Állattenyésztés és Takarmányozás*, 58. 159–172.
4. PATAKI R. – GAJDÓCSI E. – KISS R. – TEMPFLI K. – VARGA E. – KONRÁD SZ. – BALI PAPP Á. (2009): A prolaktin receptor gén alomszámra gyakorolt hatásának vizsgálata mangalica sertésekben. *Acta Agronomica Óváriensis*, 51. 73–82.
5. GAJDÓCSI E. – PATAKI R. – TEMPFLI K. – BALI PAPP Á. (2008): A prolaktin receptor gén hatása a mangalicák alomméretére. *Animal Welfare, Ethology and Housing Systems*, 4(2). 424–429.