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'THESIS OF DOCTORAL (PhD) DISSERTATION'

# HORN AND COAT COLOUR VARIETIES OF THE HUNGARIAN GREY CATTLE

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# I. INTRODUCTION

The rapidly growing human population of the last few decades resulted in the spread of world breeds with high producing ability. The increasing production of bulk goods pushed into the background the traditional domestic animal breeds, which resulted in the reduction of animal genetic resources.

Hungarian animal breeders were among the first to realize the importance of preserving animal genetic resources and took steps in order to rescue domestic animal breeds threatened by extinction. When preserving genetic resources one of the most important tasks is to maintain the typical characteristics of the breed in order to avoid loosing the available genetic variability. Therefore, traits without economic value at the moment should also be conserved. Survey and evaluation of traits forming breed character are especially important for distinguishing breeds, in present case the Hungarian Grey cattle and its closest relative, the Maremman breed, from each other.

The qualities (phenotypic and genotypic) of the Hungarian Grey cattle are subject to several research projects, however, many relationships remained unclear. Phenotype of the breed is not uniform, as the different feeding and keeping technologies and breeders' preferences formed different types. These types and the different horn and coat colour varieties which are unique characteristics of the breed have to be maintained.

One of the breed's main characteristics is the long horn which varies greatly both in shape and in colour. The colour of the coat is an important trait in forming the breed characteristics and has been used since domestication as a tool to breed formation and selection. A better knowledge of horn and coat colour varieties may help to understand the breed's history, its demographic and genetic character.

The judgement of the different coat colour varieties, due to its subjective aspect, can lead to several misinterpretations. However, the development of technology made it possible to treat this categorical trait in an objective way. For objective measurement of coat colour the Minolta Chromameter CR-410 was applied, which measures the reflectance of the light from the coat compared to a calibration plate. The L*a*b* colour space, defined by the International Commission on Illumination (CIE) in 1976 is the most widely used colour space for measuring colour of domestic animals objectively.

There is a sustained research effort to unfold the genetic background of coat colour varieties characteristic to the different animal species. Comparing to other animal species, quite less is known about the genes responsible for determining the different coat colour varieties of the cattle breeds. By combining the recent developments of molecular genetics and the results based on objective measurements, we can get a more accurate picture of the different coat colour varieties. Analyses of the genes and their polymorphisms that play a crucial role in forming coat colour enable the development of tests that make product traceability easier.

## II. AIMS OF THE RESEARCH

Aims of our research work were to survey and analyse the great variability characteristic to the Hungarian Grey cattle's horn and coat colour. Four main areas of the research work are as follows:

1. **The survey of the different horn colour varieties of the breed:**
   - separation of the different horn colours (white, 'cardy' and green) and colour varieties characteristic to the breed and determination of their ratio in the different sexes, age groups, families and bull lines
   - analysis of the extent of the black part on the horn tip.
   - relationship between the colour of the horn and the extent of the black part on the horn tip

2. **The survey of the different coat colour varieties of the breed:**
   - separation of coat colour varieties of new-born calves
   - comparison of coat colour of Hungarian Grey and Limousine calves
   - determining the ratio of coat colour varieties of adult animals
   - analysis of the relationship between subjective and objective colour judgement
   - analysis of factors influencing coat colour (sex and season of measurements)

- determining the ratio of the coat colour varieties being most characteristic to families and bull lines

3. **Analyses of the relationships among the above mentioned qualitative traits**:
   - analysis of the relationships between the coat colour of new-born calves and adult animals
   - analysis of the relationship between the colour of the horn and the coat

4. **Breed identification based on the polymorphisms of melanocortin-1 receptor gene in products made of Hungarian Grey cattle meat:**
   - determining the MC1R genotypes of some cattle breeds bred in Hungary
   - evaluating the possibilities of breed identification of raw cattle meat and processed beef products

## III. MATERIALS AND METHODS

Research work was carried out in the Hungarian Grey cattle population of the Hortobágy Non-Profit Company for Nature Conservation and Gene Preservation (which is the largest Hungarian Grey cattle stock in the world). New-born calves, heifers, cows, breeding bulls and steers were involved in the survey.

### 1. Analysis of horn colour varieties

For determining the ratio of the horn colours and colour varieties characteristic to the Hungarian Grey cattle, a subjective method was applied. The methodology was as follows: photos were taken of the animals with a digital camera, animals were identified, extent of the black part on the horn tip was determined and data were analysed by statistical programs with a computer. Photos were taken with an Olympus C500 digital camera, in a resolution of 2560x1920. A total of 670 animals (Table 1) were involved in the survey. The horn colour of breeding bulls born in the period of 1997-2003 (115 animals) was determined based on the photos present in bull catalogues.

*Table 1:*

**Number of animals**

| | Number of animals (n) |
|---|---|
| Males | 181 |
| Females (number of heifers) | 431 (180) |
| Steers | 58 |
| Total | 670 |

During the analyses of the extent of black colour on the horn tip the following categories were separated: standard horns were the ones on which the extent of black colour reached 30% of the horn's length. The horns that had smaller extent of black colour were referred to as whitened-up in the case of white horns and as smoky in the case of 'cardy' and green horns. Smoky deep down horns were the ones on which the extent of black colour exceeded 30% of the horn's length.

Distribution of the different horn colours and colour varieties were analysed by $Chi^2$-tests.

**2. Analysis of coat colour varieties**

For objective measurement of coat colour the Minolta Chromameter CR-410 was applied, which measures the reflectance of the light from the coat compared to a calibration plate. The L*a*b* colour space, defined by the International Commission on Illumination (CIE) in 1976 is the most widely used colour space for measuring colour of domestic animals objectively. The L* value shows the lightness of the colour on a scale from 0 to 100, the lower values indicating the darker colour. The a* value indicates the red/green, while the b* value indicates the yellow/blue chromaticity of the colour on a scale ranging from +60 to -60. These three colour attributes together define the position of a specified colour within a 3D colour space.

<u>Measurement of calves</u>

A total number of 303 Hungarian Grey calves from both sexes were measured during the period of January-April in 2006 and 2007. (181 calves were measured in 2006 and 122 calves in 2007). Limousine calves from both sexes were measured at the Tedej Zrt in November, 2006. Colour of the coat was measured on the left side of the body at four points.

Measurement of adult animals

Measurements of adult animals were always connected to some work (vaccination, checking of the ear-tags) performed in the stock during the period of 2005-2007. Animals were measured both in winter and summer coat.

*Table 2:*

**Number of measured animals**

| | **Number of animals (n)** |
|---|---|
| Males | 61 |
| Females | 684 |
| Steers | 183 |
| Total | 928 |

As the front and the rear part of the body are usually darker, we determined three main measurement areas: the neck-shoulder, the side and the thigh-croup area. Three-four measurements per measurement areas were made.

Discriminant analysis was used to detect the relationship between objective measurements and subjective classification. The following procedures were used in the statistical analysis: STEPDISC, DISCRIM, CLUSTER and TREE (SAS Institute, Cary, 1999). Stepwise discriminant analysis was applied to select colour variables (L*, a* and b*) and range them according to their importance. Relationship among the colour variables (L*, a* and b*) was detected by using one-way ANOVA and independent samples T-test. Relationship between the coat colour of new-born calves and the coat colour of adult animals, and relationship between horn colour and coat colour were analysed by $Chi^2$-test.

**3. Analysis of MC1R gene's polymorphisms in different cattle breeds**

Analyses were performed by using DNA extracted from blood and hair samples and the purified DNA provided by the Laboratory of Central Agricultural Office (MgSzH) *(Table 3)*. For the analysis of product traceability, different dry-products made from Hungarian Grey meat and raw cattle meat were used.

*Table 3:*

**Number of animals, type of samples and samples' origin**

| Breed | Number of animals (n) | Sample | Samples' origin |
|---|---|---|---|
| Hungarian Grey | 240, 54<br>14 | blood, hair<br>purified DNA | Hortobágy, Fertő-Hanság<br>MgSzH Laboratory |
| Hungarian Simmental | 60<br>14 | blood, hair<br>purified DNA | Derecske<br>MgSzH Laboratory |
| Charolais | 17 | hair | Léh |
| Limousine | 10 | hair | Hajdúszoboszló |
| Aberdeen angus (red-coloured) | 30<br>4 | blood, hair<br>purified DNA | MgSzH Laboratory<br>Adony |
| Aberdeen Angus (black-coloured) | 16<br>16 | blood, hair<br>purified DNA | MgSzH Laboratory<br>Adony |
| Holstein | 40<br>19 | blood<br>purified DNA | Hód-Mezőgazda Zrt., Pély-Tiszatáj Agrár Rt.,Nagykun Mg.Rt.,NarivoÁllattenyésztő és Növénytermesztő Kft.<br>MgSzH Laboratory |

The analysed samples of processed meat products were as follows: raw Hungarian Grey cattle meat, raw cattle meat (breed is unknown), Hungarian Grey cattle salami, Hungarian Grey cattle salami with paprika, sausage, stew made of Hungarian Grey cattle meat with red-wine and biosalami with paprika.

Genomic DNA was purified from blood samples by using the method of ZSOLNAI – ORBÁN (1999), and from hair samples by the method of FAO/IAEA (2004). For the analyses of listed meat products and raw meat DNA was purified by using E.Z.N.A. Tissue DNS Kit (Omega Bio-Tek., USA) according to manufacturer's protocol.

*PCR protocol*

- distilled water
- 0,2 mM dNTP mix (dATP, dCTP, dGTP, dTTP) (Pharmacia Biotech, USA)
- GoTaq Flexi DNA Ploymerase (5u/µl) (Promega, Medison, USA)
- 5x buffer (Promega, Medison, USA)
- 25 mM $MgCl_2$ (Promega, Medison, USA)
- M1 primer (10 pmol/µl) (Invitrogen Corporation, California, USA)
- M2 primer (10 pmol/µl) (Invitrogen Corporation, California, USA)
- genomic DNA (50-100 ng/µl)

**Sequences of applied primers:**

| | |
|---|---|
| **M1 (forward)** (CREPALDI et al, 2003) | 5' AAG AAC CGC AAC CTG CAC T 3' |
| **M2 (reverse)** (CREPALDI et al, 2003) | 5' GCT ATG AAG AGG CCA ACG AG 3' |

*PCR conditions*

95ºC 2 min. (initial denaturation)

95ºC 30 seconds (denaturation)
61ºC 30 seconds (annealing)
72ºC 30 seconds (extension)
} 35 cycles

72ºC 5 min. (final extension)

10ºC ∞ (cooling)

PCR was performed using a GeneAmp PCR Sytem 9700 (Applied Biosystems) thermal cycler in a volume of 20µl containing the ingredients listed above in *PCR protocol*.

PCR-amplified product size is 401bp containing the recognition sequence of restriction enzymes Msp1 and MspA1I.

PCR-RFLP was performed by using a mix in a final volume of 10µl containing 0,5µl Msp1 or MspA1I restriction enzymes (10 u/µl) (Promega, Medison, USA); 1 µl of buffer; 1,4 µl of $dH_2O$; 0,1µl of BSA and 7 µl of PCR product. This enzyme digestion mixture was incubated for 3 hours at 37 ºC. Restriction analysed fragments were separated by gel electrophoresis and stained with ethidium-bromide.

## IV. RESULTS

### 1. Analysis of horn colour varieties

Results of our survey confirmed that a great variety of horn colours characterises the Hungarian Grey cattle. Within this variety, we separated three main horn colours: the white, the green and the 'cardy' which is the mixture of the two above mentioned colours. Our results support the statement of BODÓ et al. (2002).

*Table 4:*

**Distribution of horn colours of the observed female, male and steer stocks**

| | | White | | 'Cardy' | | Green | |
|---|---|---|---|---|---|---|---|
| | **∑n** | **n** | **%** | **n** | **%** | **n** | **%** |
| **Males** | 181 | 112 | 61.88 | 46 | 25.41 | 23 | 12.71 |
| **Females** | 431 | 256 | 59.40 | 138 | 32.02 | 37 | 8.58 |
| **Steers** | 58 | 33 | 56.89 | 23 | 39.66 | 2 | 3.45 |
| **Total** | 670 | 401 | 59.85 | 207 | 30.90 | 62 | 9.25 |

Analysis of the total population (670 animals) showed that approximately 60% of the Hungarian Grey cattle stock is white-horned *(Table 4)*. The green horn which is a unique trait of the breed is characteristic to less than 10% of the surveyed population. The 'cardy'-horned animals represent 30% of the population.

Test of homogeneity showed that there are no significant differences ($P>0.05$) in the distribution of horn colours of the surveyed female and male (the critical $Chi^2$ value: 5.991; the observed $Chi^2$ value: 3.042), and the female and steer stocks (the critical $Chi^2$ value: 5.991; the observed $Chi^2$ value: 2.648). However, distribution of horn colours of the male and steer stocks were not identical ($P<0.05$). (The critical $Chi^2$ value: 5.991; the observed $Chi^2$ value: 6.259).

Our results revealed that so-called 'intermediate' horn colours can also be distinguished. Separation of these 'intermediate' horn colour varieties (white with some green; 'cardy' with a large amount of white; 'cardy' with some white; green with some white) was based on the extent of white colour. Separation of the above-mentioned horn colour varieties was not possible in the case of bulls, as the horns of males do not 'clear up' perfectly. Therefore, the data of females and steers were analysed.

*Table 5:*

**Distribution of horn colour varieties in the observed population**

| Horn colour varieties | Females | Steers | Total | Percentage |
|---|---|---|---|---|
| FAK | 35 | 7 | 42 | 26.09% |
| KSF | 54 | 6 | 60 | 37.27% |
| KKF | 40 | 9 | 49 | 30.43% |
| ZAK | 9 | 1 | 10 | 6.21% |
| Total | 138 | 23 | 161 | 100% |

*FAK: white with some green; KSF: 'cardy' with a large amount of white; KKF: 'cardy' with some white; ZAK: green with some white*

The aggregated data of the female and steer stocks showed that the ratio of horn colour varieties containing a higher extent of white colour ('cardy' with a large amount of white; 'cardy' with some white) is approximately 70%, while the ratio of the other two colour varieties: the white with some green and the green with some white (which contain only a small amount of 'foreign' colour besides the main colour) is much lower *(Table 5)*.

$Chi^2$-test of data confirmed that there are no significant differences ($P>0.05$) in the distribution of the four horn colour varieties between the female and steer stocks. (The critical $Chi^2$ value: 11.345; the observed $Chi^2$ value: 1.907).

The distribution of the black colour on the horn tip was also analysed. Results of the total population revealed the higher proportion of whitened-up and smoky horn tips in the case of all three horn colours, while ratio of standard horn tips was the lowest *(Table 6)*.

*Table 6:*

**Distribution of the black part on the horn tip in the observed population**

| White | | | 'Cardy' | | | Green | | |
|---|---|---|---|---|---|---|---|---|
| **MM** | **SZK** | **MK** | **SK** | **SZK** | **MK** | **SK** | **SZK** | **MK** |
| 43.89% | 17.96% | 38.15% | 41.06% | 24.16% | 34.78% | 56.45% | 12.90% | 30.65% |

*MM: whitened up, SZK: standard, MK: smoky deep down, SK: smoky*

Statistical analyses of data resulted in the following statement: the colour of the horn and the extent of black colour on the horn tip are proved to be without reference to each other ($P>0.01$). (The critical $Chi^2$ value: 13.277; the observed $Chi^2$ value: 6.264) (Data refer to only the female population, as data of the male and steer populations were not sufficient for performing these tests).

Distribution of horn colours in the different bull lines

Breeding of the Hungarian Grey cattle involves the use of genealogical bull lines which were first systemized by Alfonz Anker. Presently, 9 genealogical bull lines are used (A, B, C, K, M, T, V, S, L) and each bull line is named after the first capital of the founder bull's name. (For example the founder of the B line was Buda, while Maros founded the M line and so on). The S and L lines represent bull lines imported from Serbia, however there are no breeding bulls in these lines yet. The K bull line originates in the population at Bugac, the A line was part of the T line at Hortobágy before splitting.

*Table 7:*

**Distribution of horn colours in the different bull lines**

| **Bull lines** | **Horn colours** White % (n) | 'Cardy' % (n) | Green % (n) |
|---|---|---|---|
| **B** | 59.09 (13) | 18.18 (4) | 22.73 (5) |
| **C** | 73.53 (25) | 11.76 (4) | 14.71 (5) |
| **M** | 60.00 (18) | 23.33 (7) | 16.67 (5) |
| **T** | 69.56 (16) | 26.09 (6) | 4.35 (1) |
| **V** | 76.00 (19) | 12.00 (3) | 12.00 (3) |

Homogeneity test confirmed our previous hypothesis that there are no significant differences in the distribution of horn colours of the bull lines *(Table 7)*. (The critical $Chi^2$ value 9.210, the observed $Chi^2$ values between the bull lines were as follows: B-V: 0.792; B-M: 0.242; B-T: 3.358; B-C: 1.272; C-V: 0.078; M-T: 1.746; C-M: 0.039; C-T: 1.399; and M-V: 0.963).

Our results support the statements of BODÓ et al. (2002) and results of MOLNÁRNÉ et al. (2003) based on analyses of microsatellites that genealogical lines used in the breeding of the Hungarian Grey cattle have no unique traits and there are no differences between them.

## 2. Analysis of coat colour varieties

Coat colour of new-born calves

The Hungarian Grey cattle belongs to the Podolian group of cattle which is characterized by the fact that calves are born with a reddish coat colour and become grey at the age of 4-6 months. Previously, when there were only few Hungarian Grey stocks kept by excellent herdsmen, five coat colour varieties of calves were distinguished (very light, light, light reddish, reddish and dark reddish). Nowadays, this system is not used, therefore our thesis works only with three colour varieties: light reddish, reddish and dark reddish.

We found that almost half (48.52%) of the measured calf population (n=303) was reddish-coloured. The ratio of the light and the dark reddish categories were 29.04% and 22%, respectively.

*Table 8* includes the colour variables (L*, a* and b*) of the three coat colour varieties found in new-born calves. The lowest mean L* (lightness), and the highest mean a* (red/green chromaticity) and b* (yellow/blue chromaticity) values can be found in the case of the dark reddish colour variety, indicating the darker and more intensive character of this colour. The light reddish colour variety, being pigmented to the lowest extent, can be characterised by the highest mean L* and the lowest mean a* and b* values, indicating a lighter and less intensive colour.

*Table 8:*

**L*a*b* values of coat colour varieties of Hungarian Grey calves**

| | | | **Min.** | **Max.** | **Mean±SD** |
|---|---|---|---|---|---|
| light reddish | n=88 | L* | 42.12 | 84.58 | 63.23±7.63$^{A}$ |
| | | a* | 0.02 | 8.66 | 3.74±1.76$^{a}$ |
| | | b* | 3.71 | 21.78 | 12.11±3.32$^{a}$ |
| reddish | n=147 | L* | 35.43 | 69.23 | 53.15±5.05$^{B}$ |
| | | a* | 1.40 | 7.48 | 6.25±3.03$^{b}$ |
| | | b* | 5.78 | 24.28 | 15.63±2.48$^{b}$ |
| dark reddish | n=68 | L* | 33.55 | 59.58 | 46.19±4.10$^{C}$ |
| | | a* | 2.17 | 9.96 | 6.99±1.19$^{c}$ |
| | | b* | 8.84 | 20.99 | 16.00±2.02$^{b}$ |

*L* = lightness, a* =red/green chromaticity, b* = yellow/blue chromaticity*

$^{A.B.C.a.b.c.a.b.c}$*: indicate significant differences between the values (P<0.05).*

Significant differences were found among the colour variables (L*, a* and b* values) of the three distinguished coat colour varieties, except for the b* values of the dark reddish and reddish varieties.

A question came up during our analyses: are there any differences, detectable with the chromameter, between the coat colour of Hungarian Grey calves and of calves belonging to other breeds (e.g. Limousine). Therefore, Limousine calves were involved in the measurements. Analysis of the relationship among the L*a*b* values of the coat colour of Limousine and Hungarian Grey calves revealed significant differences ($P<0.05$). This statement was true for all three colour varieties of Hungarian Grey calves *(Figure 2)*. The red coat colour of Limousine calves is characterised by lower L* (lightness) and by higher a* (red/green chromaticity) and b* (yellow/blue chromaticity) values, indicating the darker and more intensive coat colour.

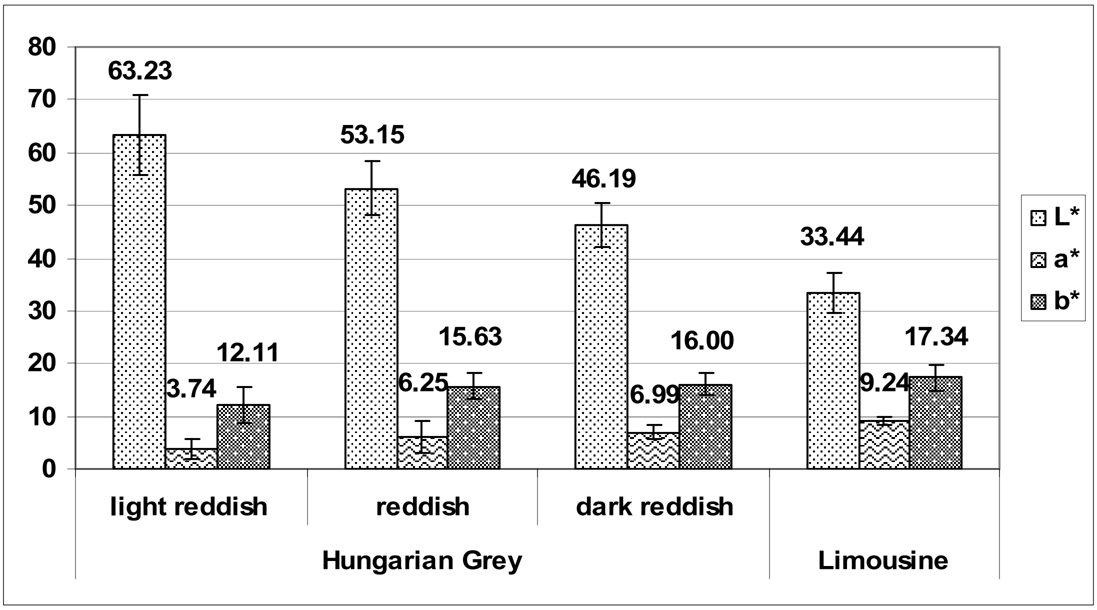


**Figure 2: Comparison of coat colour of Limousine and Hungarian Grey calves**

*L* = lightness, a* =red/green chromaticity, b* = yellow/blue chromaticity*

Coat colour varieties of adult animals

Coat colour of Hungarian Grey cattle ranges from silvery to dark crane and the colour of the bulls are usually more diversified (BODÓ et al, 2002).

*Relationship between objective measurements and subjective colour classification*

On the basis of coat colour, females were classified into seven categories: dark crane, crane, light crane, dark silvery, grey, silvery and light silvery. Our aim was to unfold the extent to which the subjective colour classification is supported by objective measurements. Moreover, colour variables (L*, a* and b*) were analysed and ranged according to their importance in separating coat colour varieties. Results showed that colour variables of all three measurement areas (neck-shoulder, side, thigh-croup) were necessary for discriminating the different coat colour varieties. However, TÓTH (2006) found that the colour variables measured on the rump are not important for separating coat colour varieties of horses. In our analyses, the L* values (lightness) proved to be the strongest discriminant factors.

Concerning the relationship between subjective colour classification and objective measurements, it can be seen that the proportion of correctly classified animals was 65%. Proportion of misclassified observations is presented in *Table 9*. Each observation is placed in the class from which it has the smallest squared distance.

*Table 9:*

**Proportion of misclassified observations**

| | **Coat colour varieties** | | | | | | |
|---|---|---|---|---|---|---|---|
| | **SD** | **D** | **VD** | **SESZ** | **SZ** | **ESZ** | **VESZ** |
| **Prior** | 0,02 | 0,09 | 0,05 | 0,19 | 0,44 | 0,16 | 0,05 |
| **Rate** | 0,71 | 0,34 | 0,33 | 0,33 | 0,36 | 0,33 | 0,27 |

*SD = dark crane, D = crane, VD = light crane, SESZ = dark silvery, SZ = grey, ESZ = silvery, VESZ = light silvery*

*Figure 3* shows the composition of posteriori clusters. For example, the crane cluster contains 65.75% of correctly classified animals. In addition, this cluster contains 19.18% light crane, 10.96% dark silvery, and 4.11% dark crane animals.

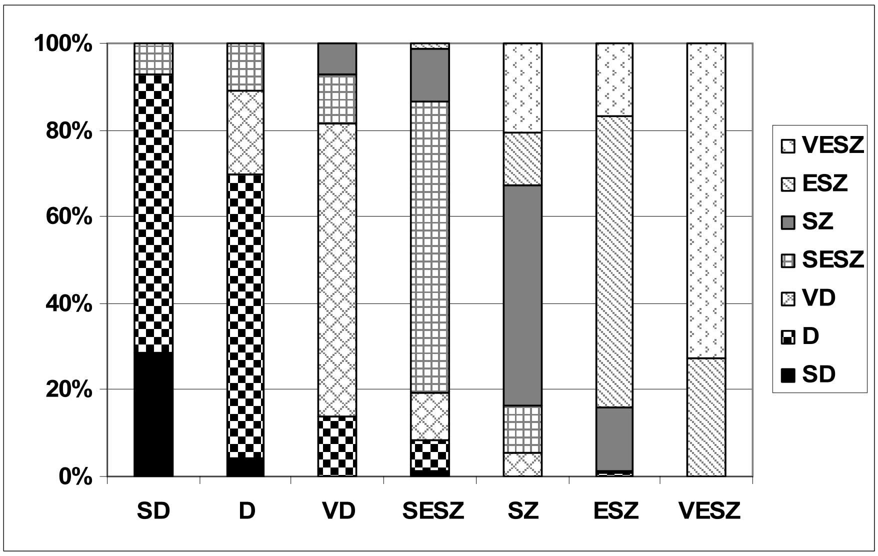


*Figure 3*: **Posteriori classification of coat colour varieties**

*SD = dark crane, D = crane, VD = light crane, SESZ = dark silvery, SZ = grey, ESZ = silvery, VESZ = light silvery*

Analyses revealed that the use of colour classification system containing seven coat colour varieties can lead to misclassifications. Therefore, we decided to combine the previously used seven coat colour classes into four (crane, grey, silvery and light silvery). (Our decision was based on results of cluster-analysis). The dark crane, crane and dark silvery classes were involved in the new crane colour class. The silvery and the light silvery classes remained as separate colour classes, while the light crane and grey classes were combined into the grey colour class.

According to this new classification system, approximately half (49.47%) of the measured female population is grey-coloured. More than quarter of the population (26.19%) is crane-coloured, while the ratio of silvery and light silvery animals is 20.69% and 7.54%, respectively.

By using this new coat colour classification system, the ratio of correctly classified animals is 75%, therefore this type of colour discrimination provides a more accurate colour judgement.

Mean L*a*b* values of the newly created coat colour classes are presented in *Table 10*.

The darker colour variety, the crane are characterised by lower L* (lightness) and by higher a* (red/green chromaticity) values than the lighter colour varieties (silvery, light silvery). Concerning the b* (yellow/blue chromaticity) value such tendency is not detectable. Our analyses revealed significant differences only among the L* (lightness) values of the three measurement areas (neck-shoulder, side, thigh-croup). In the case of a* (red/green chromaticity) and b* (yellow/blue chromaticity) values such tendency was undetectable.

*Table 10:*

**Colour variables of the new coat colour classes**

| | | **Colour variables[1]** | | |
|---|---|---|---|---|
| **Coat colour classes** | **Measurement areas** | **L*** | **a*** | **b*** |
| | | | **Mean ± SD** | |
| crane | neck-shoulder | $46.94\pm4.77^{\mathrm{A}}$ | $1.77\pm0.75^{\mathrm{a}}$ | $9.03\pm2.30^{a}$ |
| | side | $55.46\pm4.26^{\mathrm{B}}$ | $1.29\pm2.68^{\mathrm{bc}}$ | $9.41\pm2.00^{bc}$ |
| | thigh-croup | $56.53\pm4.64^{\mathrm{C}}$ | $1.28\pm0.59^{\mathrm{c}}$ | $9.51\pm1.85^{c}$ |
| grey | neck-shoulder | $54.33\pm3.44^{\mathrm{A}}$ | $1.51\pm0.73^{\mathrm{a}}$ | 10.22±2.31 |
| | side | $59.76\pm3.95^{\mathrm{B}}$ | $1.20\pm0.57^{\mathrm{b}}$ | 10.15±2.09 |
| | thigh-croup | $60.17\pm3.82^{\mathrm{C}}$ | $1.08\pm0.62^{\mathrm{c}}$ | 10.18±1.85 |
| silvery | neck-shoulder | $60.21\pm3.72^{\mathrm{A}}$ | $1.10\pm0.89^{\mathrm{a}}$ | $10.22\pm2.09^{a}$ |
| | side | $63.27\pm3.27^{\mathrm{B}}$ | $1.00\pm0.56^{\mathrm{a}}$ | $9.71\pm1.80^{b}$ |
| | thigh-croup | $63.81\pm3.11^{\mathrm{C}}$ | $0.84\pm0.59^{\mathrm{b}}$ | $10.07\pm1.76^{a}$ |
| light silvery | neck-shoulder | $64.69\pm2.88^{\mathrm{A}}$ | 0.69±0.63 | 9.64±1.63 |
| | side | $67.93\pm3.23^{\mathrm{BC}}$ | 0.67±0.65 | 9.58±1.82 |
| | thigh-croup | $68.26\pm3.04^{\mathrm{C}}$ | 0.53±0.68 | 9.59±1.71 |

[1] L* = lightness, a* =red/green chromaticity, b* = yellow/blue chromaticity
A,B,C,a,b,c,a,b,c: indicate significant differences between the values ($P<0.05$).

*Table 11.* shows the effect of season on the coat colour of females. Lower L* (lightness) values confirm the statement that the winter coat is darker in the case of all coat colour varieties characteristic to the different sexes. This statement was true for all three measurement areas. TÓTH (2006) made the same conclusions when analysing winter and summer coat of horses (Arabian Pure Bred, Shagya Arabian, Lipizzan, Gidran, Nonius). Research work of STACHURSKA et al. (2004), however, ended with contrary results: the Konik and Bilgoraj horses measured by them had lighter winter coat.

*Table 11:*

**Effect of season on the colour variables of different coat colour varieties**

| **Coat colour varieties** | **Season** | **Measurement areas** | **Colour variables**[1] L* | a* | b* |
|---|---|---|---|---|---|
| | | | **Mean ± SD** | | |
| crane | summer | neck-shoulder | 46.94±4.77$^{A}$ | 1.77±0.75$^{a}$ | 9.03±2.30$^{a}$ |
| | winter | neck-shoulder | 44.41±6.61$^{B}$ | 1.25±0.66$^{b}$ | 6.98±2.01$^{b}$ |
| | summer | side | 55.46±4.26$^{A}$ | 1.29±2.68 | 9.41±2.00$^{a}$ |
| | winter | side | 52.91±4.61$^{B}$ | 1.23±0.82 | 8.08±1.88$^{b}$ |
| | summer | thigh-croup | 56.53±4.64$^{A}$ | 1.28±0.59 | 9.51±1.85$^{a}$ |
| | winter | thigh-croup | 55.16±3.89$^{B}$ | 1.28±0.44 | 8.43±1.67$^{b}$ |
| grey | summer | neck-shoulder | 54.33±3.44$^{A}$ | 1.51±0.73$^{a}$ | 10.22±2.31$^{a}$ |
| | winter | neck-shoulder | 49.46±4.43$^{B}$ | 1.18±0.50$^{b}$ | 7.95±1.93$^{b}$ |
| | summer | side | 59.76±3.95$^{A}$ | 1.20±0.57 | 10.15±2.09$^{a}$ |
| | winter | side | 56.99±4.10$^{B}$ | 1.23±0.61 | 8.52±5.00$^{b}$ |
| | summer | thigh-croup | 60.17±3.82$^{A}$ | 1.08±0.62$^{a}$ | 10.18±1.85$^{a}$ |
| | winter | thigh-croup | 59.00±4.10$^{B}$ | 1.31±0.47$^{b}$ | 9.04±1.87$^{b}$ |
| silvery | summer | neck-shoulder | 60.21±3.72$^{A}$ | 1.10±0.89 | 10.22±2.09$^{a}$ |
| | winter | neck-shoulder | 53.13±3.59$^{B}$ | 1.15±0.73 | 8.69±1.94$^{b}$ |
| | summer | side | 63.27±3.27$^{A}$ | 1.00±0.56$^{a}$ | 9.71±1.80$^{a}$ |
| | winter | side | 58.60±4.08$^{B}$ | 1.14±0.48$^{b}$ | 8.89±1.98$^{b}$ |
| | summer | thigh-croup | 63.81±3.11$^{A}$ | 0.84±0.59$^{a}$ | 10.07±1.76$^{a}$ |
| | winter | thigh-croup | 60.56±3.90$^{B}$ | 1.21±0.39$^{b}$ | 9.35±1.74$^{b}$ |
| light silvery | summer | neck-shoulder | 64.69±2.88$^{A}$ | 0.69±0.63 | 9.64±1.63 |
| | winter | neck-shoulder | 59.11±4.52$^{B}$ | 0.60±0.69 | 9.51±1.87 |
| | summer | side | 67.93±3.23$^{A}$ | 0.67±0.65$^{a}$ | 9.58±1.82$^{a}$ |
| | winter | side | 63.18±3.56$^{B}$ | 0.96±0.43$^{b}$ | 9.16±1.67$^{b}$ |
| | summer | thigh-croup | 68.26±3.04$^{A}$ | 0.53±0.68$^{a}$ | 9.59±1.71 |
| | winter | thigh-croup | 65.18±3.37$^{B}$ | 0.83±0.53$^{b}$ | 9.47±1.57 |

[1] *L* = lightness, a* =red/green chromaticity, b* = yellow/blue chromaticity*

$^{A,B,C,a,b,c,\mathit{a,b,c}}$: *indicate significant differences between the values (P<0.05).*

### 3. Analyses of relationships between the surveyed qualitative traits

Analysis of relationship between coat colour varieties of new-born calves and adult animals

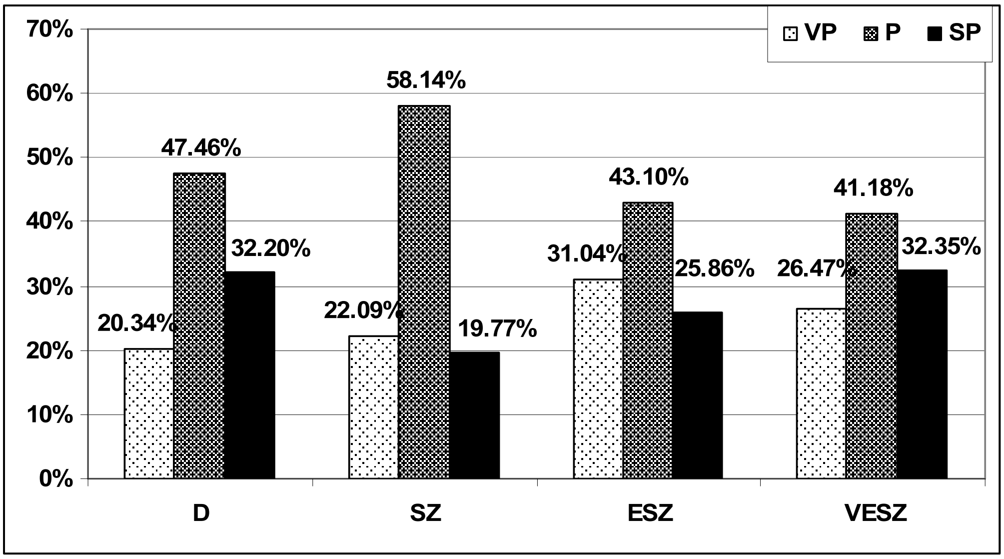


**Figure 4: Relationship between coat colour of calves and adult animals**

*D= crane, SZ= grey, ESZ= silvery, VESZ= light silvery, VP= light reddish, P= reddish, SP= dark reddish*

We found that in the case of all four coat colour varieties of adult animals, the ratio of animals born with a reddish coat colour was the highest (41.18%-58.14%), and the ratio of animals born with light reddish coat colour was the lowest (20.34%-31.04%). Results of statistical analyses confirmed that there is no significant relationship between the coat colour of new-born calves and adult animals ($P>0.01$). (Critical $Chi^2$ value: 16.812; the observed $Chi^2$ value: 6.652). That is, the coat colour variety of adult animals is not dependent on coat colour of calves *(Figure 4)*.

Analysis of relationship between the colour of the horn and the coat

We were interested in whether the darker coat colour is associated with a darker horn colour. Relationship between the two above mentioned traits is shown in *Figure 5*. High proportion of white-horned animals was found in the case of all coat colour varieties. The highest proportion (75.76%) of white-horns was found in the case of animals with light silvery coat colour. 'Cardy' horns were the second most frequent horn colours, while the ratio of green-horned animals was the lowest in all coat colour varieties.

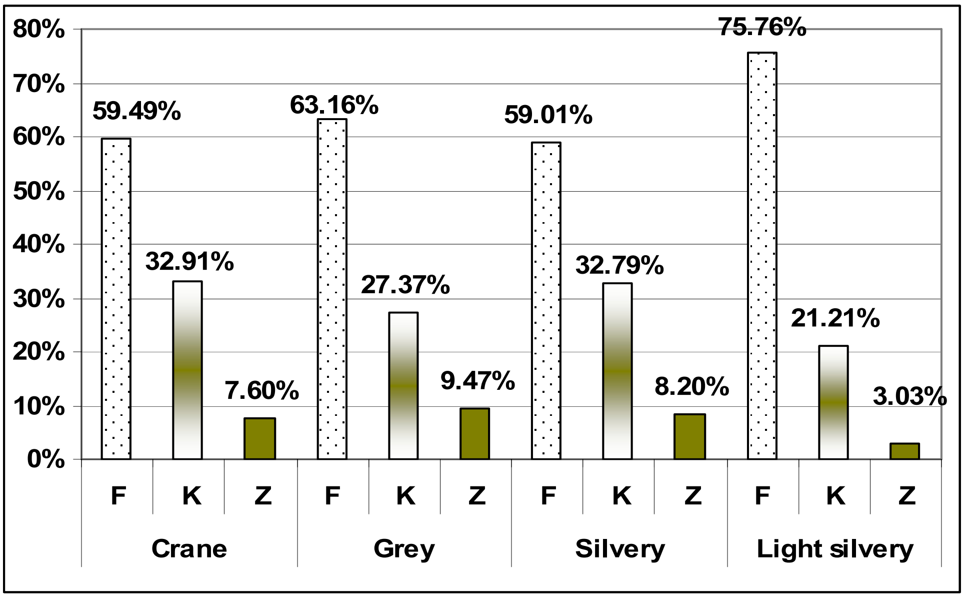


**Figure 5: Relationship between the colour of the horn and the coat**
*F= white horn, K= 'cardy' horn, Z= green horn*

Results of statistical analyses showed no statistically supportable relationship between the colour of the horn and the coat (P>0.01). (Critical $Chi^2$ value: 16.812; the observed $Chi^2$ value: 3.929). That is, these two traits are without reference to each other.

**4. Analysis of MC1R gene's polymorphisms of some cattle breeds bred in Hungary**

Pigmentation in cattle (and in general in all mammals) is determined by the presence or absence of melanins in the hair (SEARLE, 1968). The relative amounts of eumelanin (black/brown pigments) and feomelanin (yellow/red pigment) produced in melanocytes are controlled by two loci, Extension (MC1R gene) and Agouti (ASIP gene). However, several research groups (ROYO et al, 2005; GIRARDOT et al, 2005; GRAPHODATSKAYA et al, 2006) did not find any polymorphisms in the coding region of the ASIP gene of cattle breeds with different coat colours. Therefore, other genes besides the MC1R gene are suggested to be responsible for forming different coat colours.

Using PCR-RFLP we separated three alleles of the MC1R gene: a) the dominant allele, $\mathbf{E^D}$ which gives black colour, b) the recessive allele, **e** which produces red/yellow coat colour in homozygote animals and c) the wild-type allele, $\mathbf{E^+}$ which may produce a variety of colours.
Allele and genotype frequencies of the analysed breeds are shown in *Table 12*.

*Table 12:*

**Allele and genotype frequencies of the analysed breeds**

| Breed | Coat colour | Number of animals (n) | Allele frequencies | | | Genotype frequencies | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | $E^D$ | $E^+$ | e | $E^D/E^D$ | $E^D/E^+$ | $E^D/e$ | $E^+/E^+$ | $E^+/e$ | e/e |
| Hungarian Grey | grey | 308 | - | 0.997 | 0.003 | - | - | - | 0.993 | 0.007 | - |
| Hungarian Simmental | red and white | 74 | - | - | 1.00 | - | - | - | - | - | 1.00 |
| Holstein | black and white | 59 | 0.949 | 0.017 | 0.034 | 0.897 | 0.034 | 0.069 | - | - | - |
| Charolais | cream-coloured | 17 | - | - | 1.00 | - | - | - | - | - | 1.00 |
| Limousine | red | 10 | - | - | 1.00 | - | - | - | - | - | 1.00 |
| Aberdeen Angus | red | 30 | - | 0.033 | 0.967 | - | - | - | - | 0.067 | 0.933 |
| | black | 16 | 0.906 | - | 0.094 | 0.813 | - | 0.187 | - | - | - |

*$E^D$ (dominant allele): gives black colour*
*e (recessive allele): gives red/yellow coat colour in homozygote animals*
*$E^+$ (wild-type allele): produces a variety of colours*

Nowadays, 40% of the beef sold in shops originates from culled Holstein cows, therefore the most important task is to be able to distinguish products made of Holstein's and Hungarian Grey cattle's meat. All analysed Holstein animals were black-and-white-coloured, therefore they had at least one copy of $E^D$ allele. On the other hand, this dominant allele was not detectable in any of the analysed Hungarian Grey animals. Based on these results, we suggest that the analysis of MC1R polymorphisms enables the separation of the two breeds *(Table 13)*. This statement was supported by the results of analysing raw meat samples (one sample originated from Hungarian Grey cattle, while the breed of the other sample was unknown. The genotype of the control sample proved to be $E^D/E^D$, therefore we suggest it was taken from a Holstein animal).
The dominant allele ($E^D$) was found in the analysed Aberdeen Angus samples with a high frequency (0.906) and the wild-type allele (E+) was not detectable. This means that this breed is also easily distinguishable from the Hungarian Grey cattle.

Beef cattle are mainly exported from Hungary. Moreover, French beef breeds having a red (Limousine) or a diluted version of red coat colour (Charolais) were found to be fixed for allele e. Contrary to the above mentioned two French breeds, the red colour variety of the Aberdeen Angus is not easily distinguishable from the Hungarian Grey cattle, as the wild-type allele ($E^+$) is present (although with low frequency) in these animals, as well.

*Table 13:*

**Possibilities of separating breeds based on their MC1R genotypes**

| | MT | CH | AA (V) | AA (F) | HF | L | MSZ |
|---|---|---|---|---|---|---|---|
| **MT** | | – | – | + | + | – | + |
| **CH** | – | | – | + | + | – | + |
| **AA (V)** | – | – | | + | + | – | – |
| **AA (F)** | + | + | + | | – | + | + |
| **HF** | + | + | + | – | | + | + |
| **L** | – | – | – | + | + | | + |
| **MSZ** | + | + | – | + | + | + | |

*MT: Hungarian Simmental, CH: Charolais, AA (V): Aberdeen Angus, red coat colour variety, AA (F): Aberdeen Angus, black coat colour variety, HF: Holstein, L: Limousine, MSZ: Hungarian Grey, +: distinguishable from each other, -: not distinguishable from each other*

Although the wild-type allele was detectable in Simmental breeds of other countries (RUSSO et al, 2007), the Hungarian Simmental was found to be fixed for allele e. The fact that our samples originated from only two farms has to be considered when evaluating these results. The wild-type allele may be present in other populations. Identification of genetic markers responsible for forming spotting may increase the reliability of the separation of Simmental and Hungarian Grey cattle breeds.

The above statements are true only in the case of pure-bred animals.

Analyses of MC1R genotypes from processed meat products made of Hungarian Grey cattle meat

The above described PCR-RFLP method was used to analyse whether products made from Hungarian Grey cattle meat contain meat of other breeds. Based on the results presented in *Table 14*, we concluded that all analysed processed products were made only from meat of Hungarian Grey animals, as only one genotype the $E^+/E^+$, characteristic to only the Hungarian Grey breed was detectable.

*Table 14:*

**MC1R genotypes isolated from meat products**

| | **Product** | | | | |
|---|---|---|---|---|---|
| | **Salami** | **Salami with paprika** | **Sausage** | **Stew with red-wine** | **Biosalami with paprika** |
| **Genotype** | $E^+/E^+$ | $E^+/E^+$ | $E^+/E^+$ | $E^+/E^+$ | $E^+/E^+$ |

In our opinion, therefore, the analysis of MC1R gene's polymorphisms is a good tool for distinguishing products of the black and white Holstein and the Hungarian Grey cattle and for preventing any possible frauds.

## V. NEW SCIENTIFIC RESULTS

The great variability characteristic to the horn and coat colour of the Hungarian Grey cattle was analysed in my thesis. From the results of the survey performed in the period of 2004-2007, the following new scientific conclusions can be drawn:

1. It was found that **the ratio of white-horned animals is the highest** (60%) in the Hungarian Grey cattle breed. The green horn colour which is one of the most characteristic traits of the breed is present only in 10% of the animals. The ‘cardy’ horn which is a mixture of the above mentioned two colours, is characteristic to approximately 30% of the population. Statistical analyses did not support the effect of sex on horn colours. On the basis of the amount of white colour, **four additional colour varieties were distinguished within the ‘cardy’ category**. These are the followings: white with some green; ‘cardy’ with a large amount of white; ‘cardy’ with some white and green with some white. Proportions of these horn colour varieties did not differ in the female and steer populations.

2. Analysis of the **horn colour and the extent of the black colour on the horn tip** revealed that these two traits **are without reference to each other**. The highest proportion of whitened-up and smoky and the lowest ratio of standard horn tips were found in all three horn colours.

3. Based on the analysis of the coat colour of Hungarian Grey and Limousine calves, significant differences were found between the two breeds. Coat colour of Hungarian Grey calves is lighter, less reddish and more yellowish (indicated by higher L*, lower a* and higher b* values than that of Limousine calves’).

4. Results of statistical analyses confirmed that colour variables (L*, a* and b*) measured on **all three measurement areas** (neck-shoulder, side, thigh-croup) are important for separating coat colour varieties. L* values (lightness) proved to be the strongest discriminant factors.

5. **No** significant **associations** were found **between the coat colour of calves and adult animals**, and between **the colour of the horn and the extent of black colour on the horn tip**.

6. **MC1R** (melanocortin-1 receptor gene) **genotypes of** some **cattle breeds** (Hungarian Grey, Hungarian Simmental, Holstein, Charolais, Limousine and Aberdeen Angus) bred in Hungary were determined. Fixation of the wild-type allele ($E^+$) was found in the Hungarian Grey cattle. Results have shown that the Holstein and the Hungarian Grey cattle breeds **can be distinguished with high confidence** based on the polymorphisms of MC1R gene.

## VI. PRACTICAL UTILIZATION OF THE RESULTS

1. In order to maintain the great variability of horn colours characteristic to the Hungarian Grey cattle, introduction and practical application of the nominations of horn colour varieties (white with some green; ‘cardy with a large amount of white; ‘cardy’ with some white and green with some white) are suggested.

2. No differences were found among the distributions of horn colours in the different genealogical lines used in the Hortobágy population, therefore no special bull lines can be suggested for use in forming a stock with a given horn colour.

3. In the Hortobágy population, cows are classified into seven coat colour categories. However, results of the objective measurements showed that the subjective classification system can lead to several misinterpretations. For more accurate colour judgement, the use of four colour classes (crane, grey, silvery, light silvery) formed by combining the previously used seven classes is suggested.

4. Different preferences of breeders may put forward given horn or coat colour varieties or given combinations of these traits. The analyses revealed no significant relationships between these traits, which means that e.g. the coat colour of calves cannot serve as appropriate base for predicting the coat colour of adult animals. Similarly, I do not suggest the use of animals with a given coat colour for producing animals with a given coat and horn colour combination.

5. The Hungarian Grey cattle are bred mainly for preserving genetic resources of the breed, which means that the main breeding goal is the unaltered maintenance of the breed’s traits. Therefore, respect and application of the distributions of the surveyed qualitative traits are suggested.

## VII. SCIENTIFIC PUBLICATION LIST

Reviewed scientific publications

- **Radácsi A.** – Béri B. – Bodó I. (2008): Szarvszín-változatok a magyar szürke szarvasmarha fajtában. Állattenyésztés és Takarmányozás. In press.

- **Radácsi A.** – Bodó I. – Béri B. (2008): A magyar szürke szarvasmarha szarvszíneződései. Agrártudományi Közlemények. Acta Agraria Debreceniensis. In press.

- **Radácsi A. –** Bodó I. – Béri B. (2007): Újabb adatok a magyar szürke marha szőrszín-változatainak értékeléséhez. Agrártudományi Közlemények. Acta Agraria Debreceniensis. 26: 44-47.p.

- **Radácsi A.** – Bodó I. – Béri B. (2006): Szarv-és szőrszín-változatok a magyar szürke szarvasmarha fajtában. Agrártudományi Közlemények. Acta Agraria Debreceniensis. 21: 44-47.p.

- **Radácsi A.** - Bodó I. - Béri B. (2005): Different horn colour varieties in Hungarian Grey cattle. Proceedings of the 4$^{th}$ World Italian Beef Cattle Congress. Gubbio, Italy. 29. April – 1. May, 2005. 247-251.p.

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- **Radácsi A.** – Béri B. – Czeglédi L. (2008): Az MC1R gén polimorfizmusainak szerepe a szarvasmarha szőrszínének kialakításában. I. Gödöllői Állattenyésztési Tudományos Napok. Gödöllő. 11-12. April, 2008.

- **Radácsi A**. – Bodó I. – Béri B. (2006): Egyes minőségi tulajdonságok megőrzésének lehetőségei a magyar szürke fajtában. Kérődző állatfajok jelenlegi helyzete és perspektívái az Európai Unióban. Gödöllő. 10-11. April, 2006.

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- **Radácsi A**. - Tóth Zs. (2005): Újabb adatok a magyar szürke fajta génmegőrzéséhez. XI. ITF konferencia. Keszthely, 24. March, 2005.

- Tóth Zs. - **Radácsi A.** (2005): Az egyes lószínek kvantitatív megközelítése. XI. ITF konferencia. Keszthely, 24. March, 2005.

Conference proceedings in foreign languages

- **Radácsi A**. – Czeglédi L. – Szendrei Z. - Béri B. – Bodó I. (2007): The role of coat colour varieties in the preservation of the Hungarian Grey cattle. Proceedings of the 58th Annual Meeting of the European Association for Animal Production. Dublin, Ireland. 26-29. August, 2007. 307.p.

- Bodó I. – Gera I. – **Radácsi A**. – Béri B. (2007): Problems of conservation and crossing of Hungarian Grey cattle. Proceedings of the 58th Annual Meeting of the European Association for Animal Production. Dublin, Ireland. 26-29. August, 2007. 105.p.

- Bodó I. – Gera I. – Béri B. – **Radácsi. A**. (2007): Sustainability of Hungarian Grey cattle's production during centuries. Proceedings of the 58th Annual Meeting of the European Association for Animal Production. Dublin, Ireland. 26-29. August, 2007. 102.p.

- **Radácsi A**. – Béri B. – Bodó I. (2006**):** Objective measurement of coat colour varieties in the Hungarian Grey cattle. Proceedings of the 57th Annual Meeting of the European Association for Animal Production. Antalya, Turkey. 17-20.. September, 2006. 307.p.

- **Radácsi A.** (2005): Horn colour varieties in the Hungarian Grey cattle. Sustainable agriculture across the borders in Europe. Debrecen-Oradea. 06. May, 2005.

Other publications

- Czeglédi L. – **Radácsi A.** (2005): Overutilization of Pastures by Livestock. Gyepgazdálkodási Közlemények. 3. 29-35.p.