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# **Polymorphism of leptin gene (LEP/TaqI) in horses according to their breed and utility type** (Brief Report)

(Polymorphismus des Leptingens [LEP/TaqI] bei Pferden je nach Rassen- und Nutztyp)

**Background:** Leptin gene (LEP), the product of which is an important factor controlling body weight, fat reserve and feed intake, is of great interest for geneticists (XIE et al., 1999; KURYŁ, 2000).
Due to different origin of horse breeds and distinct objectives of their breeding, a question arises whether the polymorphism of leptin is going to be also characteristic for horses depending on their breed and utility type?
In the present research, investigations were undertaken with the aim to detect mutations in leptin gene (LEP) in horses bred in Poland and to determine genetic structure of horses of different breeds and utility types basing on leptin gene (LEP) polymorphism.

**Procedures:** The study included 996 horses from a range of state and private studs in Poland classified to different utility types and breeds (Table 1):

I. Warm-blooded pure-bred horses, saddle type:
   1. Pure-bred Arabians; 2. Thoroughbreds
II. Warm-blooded half-bred horses, of local breeding, saddle type:
   1. Polish noble half-bred horses, 2. Małopolski horse, 3. Wielkopolski horse
III. Warm-blooded half bred horses, of foreign breeding, saddle type:
   1. "imported horses"
IV. Warm-blooded half bred horses, of foreign breeding, light-draught type:
   1. Standardbred
V. Cold-blooded horses, heavy-draught type:
   1. Polish cold-blooded horse
VI. Primitive horses, general utility type:
   1. Polish Konik, 2. Hutsul, 3. Welsh pony + DRP, 4. Shetland pony

In order to carry out a restriction analysis of leptin gene, the methods developed by CAETANO et al. (1999) was used. The following primer sequences were applied:

5'–GTCACCAGGATCAATGACAT–3'
5'–AGCCCAGGAATGAAGTCCAA–3'

The primers flanked a DNA fragment with the size of approximately 1900 base pairs (bp) containing exons I and II separated by intron.
The PCR analysis was carried out in a 20 µl reaction mixture. This reaction mixture was composed of: single concentrated buffered solution for polymerase (50 mM KCl, 10 mM Tris-HCl), 2 mM $MgCl_2$, 200 µM nucleotide mixture (dNTP mix), 1 unit of Taq thermostable polymerase, 10 pmol of each primer, approximately 90 ng of genomic DNA. The whole reaction mixture was then supplemented with deionised water to 20 µl. The PCR thermal profile was as follows: initial denaturation of DNA matrix – 5 min at 94 °C, followed by 35 cycles: proper denaturation – 94 °C (1 min), primer annealing – 58 °C (1 min), PCR product synthesis – 72 °C (2 min) and final synthesis –

72 °C for 10 min. The obtained PCR product was digested with TaqI restriction enzyme. For this purpose, 15 µl of amplification product was incubated with 5 units of TaqI restriction enzyme (identified sequence T↓CGA) in single concentrated buffered solution Buffer TaqI+ at 65 °C for 1-2 h. The obtained restriction fragments were separated next on 2% agarose gels with addition of ethidine bromide (0.5 µg/ml) in 1×TBE buffer at 100 V, in the presence of DNA mass standard: GeneRulerTM 100 bp DNA Ladder Plus.

LEP gene analysis results were elaborated statistically using GENEPOP software package (RAYMOND et al., 1995). To characterise the genetic structure of examined horse breeds and utility types, the frequency of leptin genotypes (LEP/TaqI) together with their expected distributions and the frequency of respective alleles were calculated.

For comparing allelic and genotype frequencies between the horse breeds and utility types examined $\chi 2$ test was applied; to evaluate the significance of differences between the values obtained for particular genotypes and alleles the Duncan's multiple range test was used.

**Results:** The LEP gene amplification products were submitted to restriction analysis with the use of TaqI enzyme. The following DNA restriction fragment sizes were obtained: ~1040 and ~820 bp for genotype AA, ~1040, ~820, ~740 and ~300 bp for genotype AB, and ~820, ~740 and ~300 bp for genotype BB. The participation of respective LEP/TaqI genotypes and alleles in the horse breeds and utility types examined is presented in Table 1.

Table 1
Frequencies of genotypes and allelic of leptin gene (LEP/TaqI) in horses according to their breed and utility type (Frequenzen von Genotypen und Allelen [LEP/TaqI] bei Pferden je nach Rassen- und Nutztyp)

| Utility type | Breed | n | Genotypes LEP/TaqI | | | Alleles | |
|---|---|---|---|---|---|---|---|
| | | | AA | AB | BB | A | B |
| I | 1 | 11 | $0.091^{*}$ | 0.636 | 0.273 | 0.409 | 0.591 |
| | 2 | 8 | $0.125^{*}$ | $0.000^{*}$ | $0.875^{hjl}$ | $0.125^{*}$ | $0.875^{K}$ |
| II | 1 | 430 | $0.074^{AB}$ | $0.407^{A}$ | 0.519 | $0.278^{AB}$ | $0.722^{Ab}$ |
| | 2 | 125 | $0.072^{Cd}$ | $0.392^{b}$ | 0.536 | $0.268^{CD}$ | $0.732^{Cd}$ |
| | 3 | 40 | $0.050^{e}$ | 0.475 | 0.475 | 0.288 | $0.712^{e}$ |
| III | 1 | 70 | 0.057 | $0.329^{b}$ | $0.614^{abc}$ | $0.221^{eF}$ | $0.779^{Fg}$ |
| IV | 1 | 36 | $0.028^{*}$ | 0.278 | $0.694^{def}$ | $0.167^{g}$ | $0.833^{Hi}$ |
| V | 1 | 117 | $0.128^{F}$ | 0.504 | $0.368^{adgh}$ | $0.380^{h}$ | 0.620 |
| VI | 1 | 90 | $0.256^{dH}$ | 0.378 | $0.366^{cfkl}$ | $0.444^{i}$ | $0.556^{bdgil}$ |
| | 2 | 17 | $0.000^{*}$ | $0.294^{A}$ | $0.706^{gik}$ | $0.147^{j}$ | $0.853^{jl}$ |
| | 3 | 43 | $0.419^{Ag}$ | 0.395 | $0.186^{beij}$ | $0.616^{ACe}$ | $0.384^{ACeFHjK}$ |
| | 4 | 9 | $0.889^{BCeFgH}$ | $0.000^{*}$ | $0.111^{*}$ | $0.889^{BDFghij}$ | $0.111^{*}$ |

n – number; *n<3 for a given genotype and allele group; frequency values with the same letters in columns are significantly different at P< 0.01 (capital letter) or P<0.05 (lower case letter)

The differences occurring between the examined horses in respect of LEP/TaqI gene fragment polymorphism may be evidence of their characteristic distinction, resulting not only from their different origin but also being an effect of long-term breeding work on their predispositions for different type of use. The aforesaid studies should be continued on larger population in order to look for relationships between a specific polymorphism and horse utility value.

## References

CAETANO, A.R.; POMP, D.; MURRAY, J.D.; BOWLING, A.T.:
Comparative mapping of 18 equine type I genes assigned by somatic cell hybrid analysis. Mam. Genome **10** (1999), 271-276

KURYŁ, J.:
Livestock quantitative trait genes – current state of research, Pr. Mater. Zoot. **56** (2000), 7-50. [in Polish]

RAYMOND, M.I; ROUSSET, F.:
GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. J. of Heredity **86** (1995), 248-249

XIE, C.; ALBRECHT, E.; WEGNER, J.; BROCKMANN, G.; KAZALA, C.; WESELAKE, R.J.; ENDER, K.:
Leptin, a palatability molecule? – A Review. Arch. Tierz. **42** (1999), 191-199

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