

EFFECT OF POLYMORPHISM IN THE LEPTIN GENE ON REPRODUCTION PERFORMANCE IN PINZGAU CATTLE



Nina Moravčíková, Anna Trakovická, Radovan Kasarda

Slovak University of Agriculture in Nitra, Department of Animal Genetics and Breeding Biology,
Tr. A. Hlinku 2, 949 76 Nitra, Slovak Republic, nina.moravcikova1@gmail.com

AIM

- detection of LEP *Sau3AI* polymorphism by PCR-RFLP method,
- evaluate the relationship between the polymorphism in the leptin gene and reproduction performance in Pinzgau cattle.

MATERIAL AND METHODS

Samples were collected from 85 cows of Pinzgau breed. Genomic DNA for animal's genotyping was extracted from hair roots. A 422 bp fragment of intron 2 in bovine leptin gene was amplified by PCR using forward and reverse primers according to Liefers et al. (2002). Genotype analyses were performed using PCR-RFLP method. The PCR products of LEP gene were digested with 1 µl of FastDigest *Sau3AI* (Fermentas) restriction enzyme at 37°C in time 10 minutes. The effect of polymorphism of leptin genotypes on reproduction traits – calving interval (CI), body weight at 210 days of age (BW210), insemination interval (IN) and days open (DO) were analysed using SAS 9.1 software.

Composition of reaction mixture with a total volume of 25 µl

Component	The final concentrations
Water	-----
10 x Reaction buffer (NH ₄) ₂ SO ₄ (<i>Fermentas</i>)	1 x
MgCl ₂ 25 mM (<i>Fermentas</i>)	1,5 mM
dNTP Mix 10 mM (<i>Fermentas</i>)	2 mM
Primers (<i>Generi – Biotech</i>)	8 pM
Taq DNA polymerase 5U/µl (<i>Fermentas</i>)	1 U
BSA (<i>Fermentas</i>)	-----
Genomic DNA	50 ng

Thermal and timing of the PCR reaction

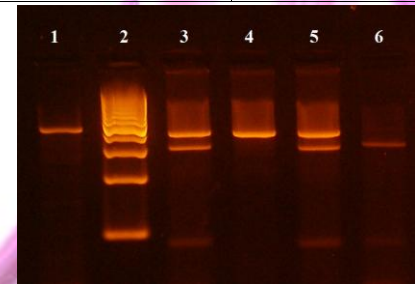
	Cycle	Temperature	Time (mm:ss)	Number of cycle
1.	„start“	95 °C	05:00	-
2.	Denaturation	95 °C	00:30	30
3.	Annealing	55 °C	00:20	
4.	Polymerization	72 °C	00:30	
5.	Elongation	72 °C	07:00	-

RESULTS

Gene and genotypic frequencies of LEP *Sau3AI* loci

Cows (n=85)	LEP <i>Sau3AI</i> loci					χ ²
	Genotype			Allele		
	AA	AB	BB	A	B	
Number	38	42	5	0.6941	0.3059	4.19
Frequency	0.447	0.494	0.059			

P>0.05



Representative result of PCR-RFLP analysis LEP *Sau3AI* loci on 3% agarose gel

Line 1 is PCR product (422 bp), line 2 is a marker of molecular weight (*Fermentas*, 100 bp), line 3 is AB genotype (390, 303, 88 and 32 bp), line 4 is AA genotype (390 and 32 bp), line 5 is AB genotype (390, 303, 88 and 32 bp) and line 5 is BB genotype (303, 88 and 32 bp)

Means and standard deviations of reproduction traits in cows of different LEP *Sau3AI*

Genotypes	n	Traits (means±SD)							
		CI (d)		BW210 (kg)		IN		DO (d)	
			N		N		N		N
<i>Sau3AI</i>									
AA	38	428.6±87.4	33	145.7±17.9	14	82.8±67.04	32	151.3±113.2	32
AB	42	384.1±90.4	30	159.8±20.4	9	96.6±93.06	38	182.5±159.1	38
BB	5	389.1±59.9	3	153.0±0	1	62.0±25.5	5	144.2±96.05	5

CONCLUSIONS

By using PCR-RFLP method have been detected genotypes in the polymorphic sites of leptin gene (*Sau3AI* loci). In the studied population of 85 Slovak Pinzgau cows were detected genotypes AA (n=38), AB (n=42) and BB (n=5). Allele A was in observed population more frequent (0.6941) than the B allele. Based on the statistical analysis SNP *Sau3AI* had significant effect only of calving interval and body weight 210 of age. The allele A seems to increase calving interval and decrease body weight 210 days of age. Non-significant associations were observed between the *Sau3AI* polymorphism and insemination index and days open.

ACKNOWLEDGMENTS

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