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TESTING OF TWO PROTOCOLS FOR GENOTYPING THE LEPTIN GENE LOCUS AND BODY MEASUREMENT IN MARAMURES BROWN AND ROMANIAN BREED CATTLE

TESTAREA A DOUA PROTOCOALE DE GENOTIPIZARE LA LOCUSUL GENEI LEPTINEI SI MASURATORI CORPORALE EFECTUATE LA RASA BRUNA DE MARAMURES SI BALTATA ROMANEASCA

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The body measurement of Maramures Brown breed and Romanian Siemmental and testing two protocols for emphasizing the leptine gene in order to perform associations with some beef production traits within further studies were the aims of our research. The blood DNA extraction was performed according to the protocols proposed by Yves Amigues and genotyping protocols was proposed by Leifers and Pomp et al. The body weight is within standards. The analyzed protocols used for leptine gene emphasizing led to satisfactory results, which will enable us to perform further research in order to make associations between this possible marker gene and some body traits.

Key words: leptine, cattle, traits, meat

Introduction

The Maramures brown breed and the Romanian Siemmental is one of Romanian cattle breeds exploited for mixed production, dairy and beef. The beef production potential of this breed in Romanian rearing conditions must be tested. According to previous studies performed in Romanian Spotted Cattle populations from Transylvanian areas, the leptine gene represents a candidate for beef production control (Vlaic, A. et al., 2001). The gene of obesity and its product, leptine was discovered in 1994. It is a proteic hormone synthesized in adipose tissues, secreted in blood, and involved in regulation of body weight, fertility, and immune function of the organism. In livestock, the control of body weight is of great importance, because the excessive development of the adipose tissues has negative influence on meat quality (Margetic, S. et al., 2002).

The body measurement of Maramures brown breed and Romanian Siemmental and testing two protocols for emphasizing the leptine gene in order to perform associations with some beef production traits within further studies were the aims of our research.

Material and Methods

The research was performed on three locations the S.C Agrozootehnica Seini, the Agricultural Association Petreşti, S.C.A. Livada, all situated in county of Satu Mare and S.C Crisan in country of Cluj. Bloods samples collection and body measurements were performed in young cattle males: 17 from S.C Agrozootehnica Seini, 34 individuals from Agricultural Association Petreşti, 18 individuals from S.C Livada, and 21 individuals from S.C.Crisan during 10.07.2007 – 30.07.2007. The following parameters were recorded: body weight, withers and croup height, oblique length, chest girth, croup width, whistle perimeter, and breast width. The measurements were performed with specific instruments (balance – body weight, meter - whistle perimeter, stick - withers and croup height, oblique length, croup width, compass - breast width).

The DNA extraction from blood was performed according to the protocols proposed by the Laboratory of Veterinary Genetics, University of California, Davis, CA 95616, modified by Yves Amigues, INRA, Jouy-en-Josas programme, and also according to the protocol for genotyping proposed by Leifers et al., 2002 and Pomp et.al 1997. Two PCR – RFLP protocols were tested in order to detect the polymorphism of the leptine gene. A 1820 DNA fragment was amplified according to Pomp et al. (1997) using the following specific primer: 5'-GTCACCAGGATCAATGACAT-3', 5' AGCCCAGGAATGAAGTCCAA 3'. In the second amplification test a 400 DNA fragment was amplified and according to Leifers(2002):5'-TGGAGTGGCTTGTTATTTTCTTCT-3'; 5'GTCCCCGCTTCT GGCTACCTAACT-3'

In both protocols, two PCR reaction mixes were used, one with 10 µl final volume, and other with 25µl final volume. The restriction of the 1820 bp PCR product was performed with *Sau 3AI* restriction enzyme at 37^0C, for 4 hours. 4% agarose gel was used for sample migration. The data were statistically processed with WINSTAT v.0.6.fig 1 (a si b).

Results and Discussions

The results of the body measurement performed in Maramures brown breed analyzed young cattle populations are presented in tables 1, 2 and 3.

The measurement performed in young cattle from S.C Agrozootehnica Seini revealed an average body weight of 338.82 kg, and coefficient of variability recorded big variations between 5.95% in croup height and 30.29 % in body weight (table 1). The young cattle reared at S.C Livada Satu Mare recorded an average body weight of 253.61 kg, chest width 0.57 m, and coefficient of variability

recorded values between 7.48 % in croup height and 22.06 % in body weight (Table 2).

Table 1. Body measurements traits recorded in young Maramures brown breed individuals from S.C Agrozootehnica Seini

Traits	n	X mediu	±	SX	s	V%
Body weight kg	17	338.82	±	24.90	102.65	30.29
Withers height m	17	1.20	±	0.02	0.09	7.26
Croup height m	17	1.29	±	0.02	0.08	5.95
Oblique length of the trunk m	17	1.37	±	0.04	0.16	11.85
Thorax depth m	17	0.60	±	0.02	0.06	10.47
Croup width m	17	0.42	±	0.01	0.05	10.98
Chest width m	17	0.60	±	0.01	0.13	11.18
Thorax perimeter m	17	1.67	±	0.05	0.19	11.31
Leg perimeter m	17	0.19	±	0.01	0.02	11.82

Table 2. Body measurements traits recorded in young Maramures brown breed individuals from S.C Livada Satu Mare

Traits	n	X mediu	±	SX	s	V%
Body weight kg	34	253.61	±	13.18	55.94	22.06
Withers height m	34	1.13	±	0.02	0.10	8.70
Croup height m	34	1.21	±	0.02	0.09	7.48
Oblique length of the trunk m	34	1.25	±	0.02	0.10	8.02
Thorax depth m	34	0.57	±	0.01	0.04	7.91
Croup width m	34	0.36	±	0.02	0.08	20.68
Chest width m	34	0.59	±	0.02	0.14	9.27
Thorax perimeter m	34	1.50	±	0.03	0.14	9.07
Leg perimeter m	34	0.16	±	0.00	0.02	12.05

Table3. Body measurements traits recorded in young Maramures brown breed individuals from S.A Petreşti, Satu Mare

Traits	n	X mediu	±	SX	s	V%
Body weight kg	13	276.18	±	14.13	82.38	29.83
Withers height m	13	1.13	±	0.02	0.10	8.47
Croup height m	13	1.18	±	0.03	0.20	17.31
Oblique length of the trunk m	13	1.24	±	0.05	0.29	23.11
Thorax depth m	13	0.59	±	0.01	0.06	10,45
Croup width m	13	0.36	±	0.01	0.07	18.47
Chest width m	13	0.56	±	0.01	0.11	13.37
Thorax perimeter m	13	1.53	±	0.03	0.18	11.79
Leg perimeter m	13	0.17	±	0.00	0.02	10.87

Table 4. Body measurements traits recorded in young Romanian Siemmental breed individuals from S.C Crisan Gherla

Traits	n	X mediu	±	SX	s	V%
Body weight kg	21	269.000	±	16.794	76.960	28.610
Withers height m	21	1.094	±	0.018	0.083	7.572
Croup height m	21	1.177	±	0.017	0.079	6.706
Oblique length of the trunk m	21	1.235	±	0.029	0.131	10.602
Thorax depth m	21	0.320	±	0.008	0.038	11.942
Croup width m	21	0.361	±	0.010	0.047	13.051
Chest width m	21	0.570	±	0.034	0.155	10.508
Thorax perimeter m	21	0.163	±	0.003	0.013	8.271
Leg perimeter m	21	0.421	±	0.003	0.013	3.188

The main body measurements traits recorded in young Maramures Brown individuals from S.A Petreşti, Satu Mare (table 3) revealed values of 276.18 kg body weight, 0.59 m breast width, high variability concerning body weight (29.83 %). I other measured parameters the coefficient of variability recoded big values too, between 8.47 % (girth height) and 23.11 % (oblique length).

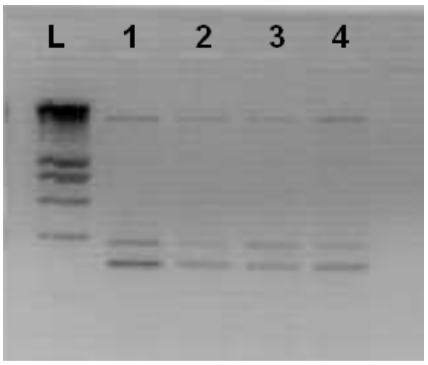
The measurement performed in young cattle from Romania Siemmental from S.C Crisan Gherla revealed an average body weight of 269 kg, and coefficient of variability recorded big variations between 6,706 % in croup height and 28,61 % in body weight (table 4).

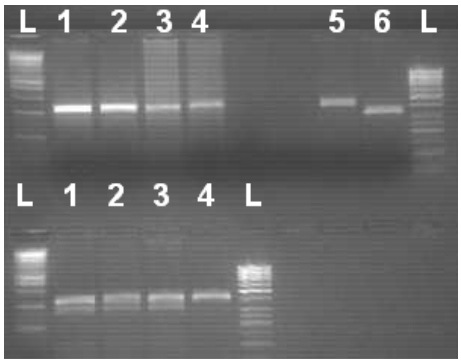
Fig.1. The tested DNA analyzes protocols proposed by Pomp (a) and Leifers (b), respectively, led to satisfactory results making possible the discrimination between the most common A and B allele in this locus.

a) line1, line2,line3 and line 4 the PCR cut product with SAU 3AI

b) line 1, line 2,line 4 and line 4, the PCR ucut product product

a) b)





The average body weight recorded in analyzed young Maramures Brown individuals and Romanian Siemmental 253.61 kg. 276.18 kg and 338.22 kg and 269.00 is within standards stipulated by international normative. 250 – 360 kg. The other analyzed body measurement parameters recorded in young individuals are within standards (e.g. withers height 1.10 – 1.30 m, croup height 1.15 – 1.30 m, croup width 0.30 – 0.55 m, whistle perimeter 0.15 / 0.21 m, chest girth 1.50 – 1.72 m, chest width 0.50 – 0.73 m, oblique length 1.20 – 1.40 m). Further research is needed in order to elaborate a real biometric picture of the present. Maramures brown and Romanian Siemmental is into Transylvanian area. The AA genotype fragments of 400 pb and the AB genotype fragments of 400, 300, and 100 pb of which of the latter is not visible in this gel.

The analyzed protocols used for leptine gene emphasizing, led to satisfactory results, which will enable us to perform further research in order to make associations between this gene and body traits.

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