

Animal welfare, etológia és tartástechnológia



Animal welfare, ethology and housing systems

Volume 9

Issue 3

Különszám/Special Issue

Gödöllő
2013



THE EFFECT OF LEPTIN GENE ON BEEF EFFICIENCY OF CZECH FLECKVIEH CATTLE

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Abstract

Leptin is a hormone affecting the regulation of body composition, energy balance and meat quality in mammals. The objective of this study was to evaluate the association of single nucleotide polymorphisms in coding region for leptin gene with carcass and beef quality of Czech Fleckvieh bulls (n = 75). Three experimental groups of these animals were created depending on different leptin genotypes (CC, n=25; TC, n=26 and TT, n=22). Bulls with TT genotype had the lowest ($p < 0.05$) content of intramuscular fat ($1.77 \pm 0.13\%$) in beef. The content of total protein (CC = 21.25; CT = 21.20; TT = 20.87%) and collagen (CC = 2.57; CT = 2.27; TT = 2.42 g.100g⁻¹) was not affected ($p > 0.05$) by leptin gene, but there was significant effect of leptin gene on the colour of beef. Meat had the brightest red colour ($L^* = 33.87\text{--}36.77$; $a^* = 9.03\text{--}10.12$; $b^* = 7.95\text{--}9.17$), which is desirable for buyers, consumers.

Key words: Czech Fleckvieh cattle, leptin, beef quality

Introduction

Meat quality is of great importance to the beef industry where the consumer is willing to pay more for superior products (*Shackelford et al. 2001; Dekkers and Hospital, 2002*). Traditionally trait improvement in livestock has used quantitative genetics theory to determine animals with high genetic merit. This selection approach is most effectively implemented for highly heritable traits that are easily measured. Meat quality traits can usually only be measured post-slaughter and often have low heritabilities (*Gill et al., 2010*). Marker assisted selection has the potential to increase the rate of genetic improvement. Markers found in various candidate genes linked to economically relevant traits have been identified and incorporated into commercially available genetic tests for meat quality. The leptin gene located on bovine chromosome 4, encodes leptin, a peptide hormone, which is synthesized and secreted by adipose tissue (*Zhang et al. 1994*). Leptin is a hormone affecting the regulation of body composition, energy balance and meat quality in mammals (*Tian et al. 2013*). The concentration of leptin could be seen as an indicator of marbling, back fat depth and yield and quality grade in feedlot cattle (*Geary et al. 2003*). Markers at promoter regions of the bovine leptin gene have been shown to be associated with carcass and meat quality traits (*Nkrumah et al. 2005*).

The aim of this study was to evaluate associations of leptin gene on beef quality traits in Czech Fleckvieh cattle.



Material and methods

The experiment was carried out on beef farm in southern Moravia, the Czech Republic. The experimental animals, 75 pure-bred Czech Fleckvieh bulls, were loose-housed in pens with slatted floors from the age of 6 months till slaughter. Three experimental groups of these animals were created depending on different leptin genotypes (CC, n=25; TC, n=26 and TT, n=22). Blood samples (2 ml) were collected into tubes with EDTA. Blood was stored at -20 °C. Genomic DNA was isolated from blood using the QIAamp DNA Blood Mini Kit (Qiagen Inc., Valencia, CA, USA). The quality of DNA was verified by agarose gel electrophoresis in 1% gel visualized with ethidium bromide. Genotypes were determined based on molecular genetic analysis of single-nucleotide polymorphism (SNP) in the exon 2 of the leptin gene (transition C → T) (Buchanan et al., 2002). For testing, we used our own methodology. PCR primers were designed based on GenBank U50365 sequence (FW: 5'TCGTTGTTATCCGCATCTGA 3', REV: 5'TACCGTGTGTGAGATGTCATTG 3'). The PCR was performed in 12.5 µl volumes containing 25 ng of bovine genomic DNA, 1x HotStarTaq Master Mix (Qiagen) and 0.2 µM of each forward and reverse primer. A PCR thermal profile consisted of pre-denaturation at 95 °C for 2 min; followed by 30 cycles of denaturation at 95°C for 30 s, annealing at temperature 56°C for 30 s, elongation at 72°C for 30 s; and final extension at 72°C for 7 min. The obtained PCR products of 278 bp in size were verified on 3% agarose gel and resequenced using the ABI PRISM 3100-Avant Genetic Analyzer. The polymorphic locus (C/T) is located at position 204 of the fragment. Genotypes were determined based on the sequence. They were fed an identical feed ratio based on an ad libitum intake of maize silage and a limited amount (3 kg per bull per day) of a concentrate which consisted of 50% crushed barley and 50% of soya meal with a mineral and vitamin additive. Bulls were slaughtered at the age of 620 days. The average weight gain during fattening was 822 ± 108 g per day. The carcasses (weight ranged from 395 to 463 kg) were classified to the class of meatiness “U” and “R” and the class of fattiness “2”. The beef samples (*musculus longissimus lumborum et thoracis*) were excised from carcass at the half cutting level between 9th and 10th rib and analysed. For chemical analyses, dry matter was determined in 5 g samples pre-dried at 60 °C for 2 h, mixed with dry sea sand and dried at 105 °C for 6 h. Ash content was measured gravimetrically after burning the sample (2 g) in a muffle laboratory oven (LMH 11/12; LAC Rajhrad, Czech Republic) at 550 °C for 8 h. Protein (Kjeldahl nitrogen×6.25) and collagen content was determined according to the AOAC methods (2000 and 1996, respectively). Myoglobin concentration was measured as described by Hornsey (1956). Intramuscular fat (IMF) was determined gravimetrically after spiking 50 g of the MQF sample with 5 ml of the internal standard solution (2.5 mg of C15:0/ml isooctane; Supelco) and extraction with petrol ether in a Soxhlet extractor for 6 h. Water holding capacity was determined in 2 g of the MQF samples according to Honikel (1998). Meat colour was estimated based on the L*a*b* system (lightness, redness, yellowness; Centre Internationale de l'Eclairage, 1976) using a Konica Minolta CM-2600d spectrophotometer containing an integrated spectral component, a D65 illuminator and a 10° observer. Cut surface was exposed to the air at ambient temperature (blooming) for 1 h before the colour was measured. Experimental data were assessed using STATISTICA software, version 10.0 (StatSoft, Inc., Tulsa, Oklahoma, USA), where the genotypic effects of leptin gene on meat quality parameters was carried out using the GLM procedure.



Results and discussion

The chemical analysis of the MLT (*Table 1*) revealed several significant differences between leptin genotypes. The TT samples had the lowest content of dry matter ($26.14 \pm 0.11\%$). A significantly ($p < 0.05$) higher level of dry matter ($27.03 \pm 0.27\%$) was found in CT genotype. Content of the total protein was from 20.87% (TT genotype) to 21.25% (CC genotype). No differences in protein content were observed by *Velik et al.* (2008) between Fleckvieh and Fleckvieh x Charolais. *Bartoň et al.* (2010) cite the same content of protein in the Czech Fleckvieh bulls ($21.18 \pm 1.3\%$). The lowest ($p > 0.05$) rate of collagen ($2.57 \pm 0.15 \text{ g} \cdot 100\text{g}^{-1}$) was detected in beef Czech Fleckvieh cattle with CC genotype for leptin gene. *Dubost et al.* (2013) published lowest content of collagen in beef. Authors presented significant differences ($p < 0.05$) among Aberdeen Angus ($4.69 \text{ g} \cdot 100\text{g}^{-1}$), Limousine ($3.73 \text{ g} \cdot 100\text{g}^{-1}$) and Blond d'Aquitaine ($3.61 \text{ g} \cdot 100\text{g}^{-1}$). The same results have already been shown by *Christensen et al.* (2011) and *Jurie et al.* (2011). Significant ($p < 0.05$) differences were approved between genotype CT (2.51%) and CC (1.84%), respectively CT (2.51%) and TT (1.77%) in intramuscular fat (IMF) content. *Scollan et al.* (2006) claim that lean beef has low IMF content typically 2–5% and in many countries this is regarded as being “low in fat”. *Nürnberg et al.* (2005) found out no differences in IMF contents between German Holstein and German Simmental bulls. By contrast, the differences in the IMF content caused by breed were reported a.g. by *Bureš et al.* (2006) and *Cuvelier et al.* (2006).

Table 1: Nutritional quality traits in Czech Fleckvieh bulls

| Trait | Genotypes | | | | | |
|-----------------------------------|--------------------|------|--------------------|------|--------------------|------|
| | CC | | CT | | TT | |
| | LSM | ± SE | LSM | ± SE | LSM | ± SE |
| Dry matter (%) | 26.43 ^a | 0.15 | 27.03 ^b | 0.27 | 26.14 ^a | 0.11 |
| Intramuscular fat (%) | 1.84 ^a | 0.16 | 2.51 ^b | 0.33 | 1.77 ^a | 0.13 |
| Total protein (%) | 21.25 | 0.14 | 21.20 | 0.15 | 20.87 | 0.15 |
| Collagen (g.100 g ⁻¹) | 2.57 | 0.15 | 2.27 | 0.12 | 2.42 | 0.08 |
| Ash (%) | 1.07 | 0.01 | 1.05 | 0.01 | 1.08 | 0.01 |

a, b: means with different superscripts are significantly different at $p < 0.05$

**Table 2: Technological quality traits in Czech Fleckvieh bulls**

| Trait | Genotypes | | | | | |
|---------------------------------|--------------------|------|--------------------|------|--------------------|------|
| | CC | | CT | | TT | |
| | LSM | ± SE | LSM | ± SE | LSM | ± SE |
| Water holding capacity (%) | 81.97 ^a | 1.14 | 81.39 | 0.83 | 79.01 ^b | 0.51 |
| pH ₄₈ | 5.79 | 0.09 | 5.71 | 0.06 | 5.62 | 0.05 |
| Myoglobin (mg.g ⁻¹) | 3.84 ^a | 0.14 | 3.63 | 0.11 | 3.45 ^b | 0.11 |
| L* | 33.87 ^A | 0.76 | 34.51 ^A | 0.63 | 36.77 ^B | 0.53 |
| a* | 9.03 ^a | 0.37 | 9.89 | 0.30 | 10.12 ^b | 0.42 |
| b* | 7.95 ^A | 0.36 | 7.99 ^A | 0.31 | 9.17 ^B | 0.29 |

a, b: means with different superscripts are significantly different at $p < 0.05$, respectively A, B: means with different superscripts are significantly different at $p < 0.01$

The TT samples had the lowest ($p < 0.05$) content of water holding capacity ($79.01 \pm 0.51\%$) than meat from group of bulls with genotype CC ($81.97 \pm 1.14\%$). The same trend, declining value from genotype CC (5.79) to TT (5.62), was found in pH₄₈. Tian et al. (2013) approved significant ($p < 0.05$) differences in pH of beef from Simmental bulls between genotype TT and CC. Accelerated pH decline is associated with the development of low water holding capacity (Huff-Lonergan and Lonergan, 2005). Statistically significant effect of leptin genotypes on the colour of beef was found. The brightest red meat was obtained from genotype TT ($L^* = 36.77 \pm 0.53$; $a^* = 10.12 \pm 0.42$) and the darkest meat from genotype CC ($L^* = 33.87 \pm 0.76$; $a^* = 9.03 \pm 0.37$). The same trend was found in yellowness (b^*) value (CC = 7.95 < CT = 7.99 < TT = 9.17). A comparable results of the colour of beef evaluation were reported by Huuskonen et al. (2010). Li et al. (2013) indicated no significant ($p > 0.05$) effect of leptin gene on colour of beef. Kadim et al. (2004) and Weglarz (2010) reported relationships between the value of colour parameters L^* , a^* and b^* in different slaughter seasons of the year.

Conclusions

The effect of leptin gene on beef quality was proved in Czech Fleckvieh cattle population. Bulls with TT genotype had the lowest content of intramuscular fat in beef, meat had the brightest red colour, which is desirable for buyers, consumers. The content of the total protein and collagen was not affected by leptin gene, but there was a significant effect of leptin gene on the colour of beef.

Acknowledgements

This study was supported by project No. QI91A055 which is financed by the Ministry of Agriculture of the Czech Republic.



References

- Bartrouň, L., Bureš, D., Kudrna, V.* (2010): Meat quality and fatty acid profile of the musculus longissimus lumborum in Czech Fleckvieh, Charolais and Charolais x Czech Fleckvieh bulls fed different types of silages. *Czech Journal of Animal Science*, 55. 479-487.
- Buchanan, F.C., Fitzsimmons, C.J., Van Kessel, A.G., Thue, T.D., Winkeman-Sim, D.C., Schmutz, S.M.* (2002): Association of a missense mutation in the bovine leptin gene with carcass fat content and leptin mRNA levels. *Genetics Selection Evolution*, 34. 105-116.
- Dekkers, J.C.M., Hospital, F.* (2002): The use of molecular genetics in the improvement of agricultural populations. *Nature Reviews. Genetics*, 3.22-32.
- Dubost, A., Mocol, D., Meunier, B., Lethias, C., Listrat, A.* (2013): Relationships between structural characteristics of bovine intramuscular connective tissue assessed by image analysis and collagen and proteoglycan content. *Meat Science*, 93. 378-386.
- Honikel, K.O.* (1998): Reference methods for the assesment of physical characteristics of meat. *Meat Science*, 49. 447-457.
- Hornsey, H.C.* (1956): The color of cooked cured pork. 1. Estimation of the nitric oxidehaem pigments. *Journal of the Science of Food nad Agriculture*, 7. 534 - 540.
- Huff-Lonergan, E., Lonergan, S.M.* (2005): Mechanisms of water-holding capacity of meat: the role of postmortem biochemical and structural changes. *Meat Science*, 71. 194 - 204.
- Christensen, M., Ertbjerg, P., Failla, S., Sanudo, C., Richardson, R.I., Nute, G.R., Olleta, J.L., Panea, B., Alberti, P., Juarez, M., Hocquette, J.F., Williams, J.L.* (2011): Relationship between collagen characteristics, lipids contentand raw and cooked texture of meat from young bulls of fifteen european breeds. *Meat Science*, 87. 61 - 65.
- Geary, T.W., McFadin, E.L., MacNeil, M.D., Grings, E.E., Short, R.E., Funston, R.N., Keisler, D. H.* (2003): Leptin as a predictor of carcass composition in beef cattle. *Journal of Animal Science*, 81. 1-8.
- Gill, J.L., Bishop, S.C., McCorquodale, C., Williams, J.L., Wiener, P.* (2010): Associations between single nucleotide polymorphisms in multiple candidate genes and carcass and meat quality traits in a commercial Angus-cross population. *Meat Science*, 86. 985 - 993.
- Huuskonen, A., Jansson, S., Honkavaara, M., Tuomisto, L., Kauppinen, R., Joki-Tokola, E.* (2010): Meat colour, fatty acid profile and carcass characteristics of Hereford bulls finished on grazed pasture or grass silage-based diets with similar concentrate allowance. *Livestock Science*, 131. 125 - 129.
- Jurie, C., Picard, B., Micol, D., Listrat, A., Kammoun, M., Horcquette, J.F.* (2011): Muscle characteristics from young bulls of different beef breeds. In 57th ICoMST, M. Science, Ghent, Belgique, 243 - 324.
- Kadim I.T., Mahgoub, O., Al-Ajmi, D.S., Al-Maqbaly, R.S., Al-Mugheiry, S.M., Bartolome, D.Y.* (2004): The influence of season on quality characteristics of hot-boned beef m. longissimus thoracis. *Meat Science*, 66. 831 - 836.
- Nkrumah, J.D., Hansen, C., Keisler, D.H., Li, C., Irving, B., Wang, Z., Moor, S.S.* (2005): Relationship between serum leptin concentration and BW, feed intake, ultrasound traits and carcass merit of hybrid beef cattle. *Journal of Dairy Science*, 88. 167 - 167.
- Shackelford, S.D., Wheeler, T.L., Meade, M.K., Reagan, J.O., Byrnes, B.L., Koohmaraie, M.* (2001): Consumer impressions of Tender Select beef. *Journal of Animal Science*, 79, 2605-2614.



- Tian, J., Zhao, Z., Zhang, L., Zhang, Q., Yu, Z., Li, J., Yang, R. (2013): Association of the leptin gene E2-169T>C and E3-299T>A mutations with carcass and meat quality traits of the Chinese Simmental-cross steers. *Gene*, 518. 443 – 448.
- Velik, M., Steinwiddler, A., Frickh, J.J., Ibi, G., Kolbe-Romer, A. (2008): Effect of ration, sex and breed performance and meat quality of cattle from suckler cow systems. *Zuchtungskunde*, 80. 378 – 388.
- Weglarz, A. (2010): Meat quality defined based on pH and colour depending on cattle category and slaughter season. *Czech Journal of Animal Science*, 55, 548 - 556.
- Zhang, Y.J., Proenca, R., Maffei, M., Barone, M., Leopold, L., Friedman, J.M. (1994): Positional cloning of the mouse obese gene and its human homologue. *Nature*, 372. 425–432.