

Relations of the leptin gene polymorphism and some internal environment parameters

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Abstract: The aim of this study was to evaluate effects of the single nucleotide polymorphism for leptin gene on selected metabolic parameters of Czech Pied cattle bulls. Missense mutation (cytosine-thymine switch) results in meat quality increase considering not only cattle but also another livestock. Animals with genotype TT distinguishes with higher leptin concentration in blood. These animals has higher amount of intramuscular fat (marbling score) in comparison to the animals with genotype CC. The leptin does influence the metabolism of lipids and energy, therefore the metabolic parameters of blood were observed and compared to genotypes. The relation between concentration of creatinine and genotype TT was found to be statistically significant ($P < 0.05$), though other relations of parameters (glucose, urea, albumin) and genotypes were not.

Key-Words: SNP leptin, energy metabolism, urea, albumin, creatinine

Introduction

Leptin is the proteinaceous hormone produced by obese gene and synthesized by adipocytes. Leptin does circulate in blood in both free form and bound form [16] and it also shows retroactive effects [1]. There is influence of leptin on the regulation of the energetic metabolism [14]. It does signalize the amount of energetic provisions in periphery to the central nervous system [13]. The increase of leptin concentration in blood plasma stimulates leptin receptors located in hypothalamus, which does subsequently inhibit food intake and stimulates energy expenditure [7, 1]. Leptin does interfere with energy metabolism in peripheral tissues and probably influence insulin in adipocytes and muscles [3].

Leptin comprises 167 amino acids as a product of obese gene. The leptin gene was mapped at 4th chromosome in the case of cattle. It consists of three exons. The second exon bears point mutation (C/T), therefore the coding amino acid is changed from arginine to cysteine [4]. The genotype TT of animal does result in qualitative advantage of meatpacking parameters. These individuals indicates larger amount of intramuscular fat (marbling score) to the individuals distinguished by CC or CT, there is usually also higher evaluation in the means of body score condition. Single nucleotide polymorphism (SNP) is associated with

the leptin concentration in serum [12] and amount of the stored fat in organism [3]. The level of leptin in blood or the amount of mRNA in fat tissue is in relation with body mass, food intake and body fat [5]. The similar research was performed on another livestock species, the leptin gene mutation correlates with the height of dorsal fat observed in pigs [15]. Leptin is engaged in processes of energy expenditure, food intake regulation, regulation of hormones secretion of endocrine system, reproduction, immune response and renal function [8].

Material and Methods

Animals

The experiment was performed on the Czech Pied bulls in age of 240 days. The animals were sorted out into three groups according to the leptin genotype. The genotype TT was represented by 18 animals, TC was represented by 133 animals and CC was represented by 102 animals. Animals were breed under identical conditions and fed with the same feeding ration based on the maize silage.

Genetic analysis

The blood samples were taken from animals into the test tube with anticoagulant agent EDTA. The samples were stored in the environment of -20° Celsius until the examination. The genome DNA of

animals was isolated by the means of QIAamp DNA Blood Mini Kit (Qiagen Inc., Valencia, CA, USA). The quality of isolated DNA was verified by the electrophoresis on 1% agarozoidal gel with ethidium bromide. The genotypes were differentiated on the basis of single nucleotide polymorphism in second exon of the bovine leptin gene. For testing, we used our own methodology. PCR primers were designed based on the nucleotide sequence of bovine leptin gene (GenBank U50365) (FW:5'TCGTTGTTATCCGCATCTGA3', REV: 5'TACCGTGTGTGAGATGTCATTG 3'). PCR were carried out in 12.5 µl volume containing 25 ng of cattle genome DNA, 1x HotStarTaq Master Mix (Qiagen) and 0.2 µM of straight and reverse primer. There is a multi-step process of PCR. 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 56°C for 30 s, elongation at 72°C for 30 s; and final extension at 72°C for 7 min. The PCR products of 278 bp in size were separated on 3% agarose gel and sequenced using the ABI PRISM 3100-Avant Genetic Analyzer. The polymorphic locus (C/T) is located at position 204 base of the fragment.

Blood samples

The blood samples for biochemical analysis were taken from *vena jugularis externa*. All the bulls were sampled from 8:00 am to 9:30 am. Test tubes equipped with silicone separating gel and coagulation accelerator (Dispolab, Czech Republic) were used. The serum was separated by centrifugal machine set to 2,000 g for 10 minutes at 4° Celsius and further stored at temperature -20° Celsius.

Biochemical analysis

Urea, albumin, creatinin and glucose were analyzed by the Konelab T20xt (Thermo Fisher Scientific, Finland) using commercial kits (Biovendor-Laboratorní medicína, Czech Republic).

Statistical analysis

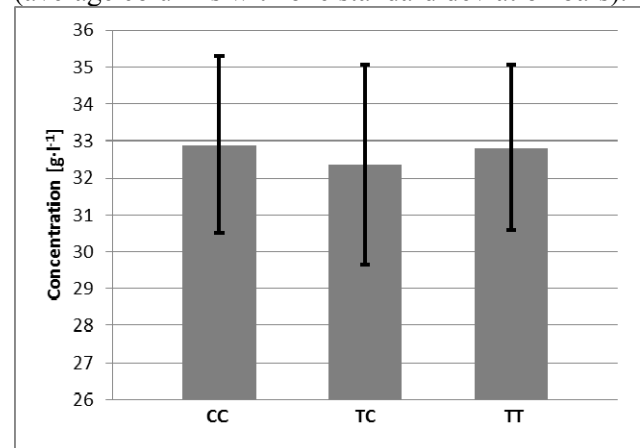
Measured data were sorted out and the univariate statistical analysis based on normal distribution was used in order to describe expected values and its dispersion characteristics within Czech Pied cattle population. The levels of significance of resulting differences were considered by the means of bivariate statistical analysis (Student's tests).

Results and discussion

Leptin was reported to be a potent regulator of food intake. There was evidence indicating that at least some of the effects of leptin occurred through

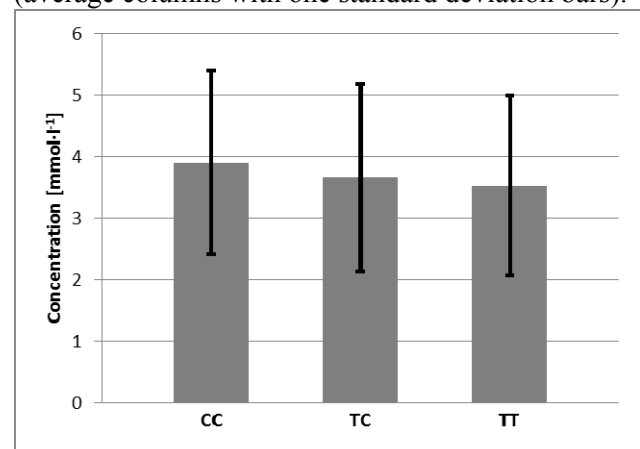
receptor-mediated regulation of the hypothalamic protein neuropeptide Y (NPY), which was a potent stimulator of food intake [5].

Fig. 1 Relation of genotype of Czech Pied bulls for leptin gene and level of albumin in blood serum (average columns with one standard deviation bars).



Nutritional status, corresponding with food intake in animals could be evaluated by measurements of some biochemical markers such as plasmatic concentration of albumin. Several studies [6] were addressed to the relationship between plasma leptin concentration and nutritional status. But they did not find a significant correlation between leptinemia and plasma concentrations of albumin. These ascertainments corresponded with the results of our study. The albumin concentration in blood plasma in relation to leptin genotype was not found to be statistically significant.

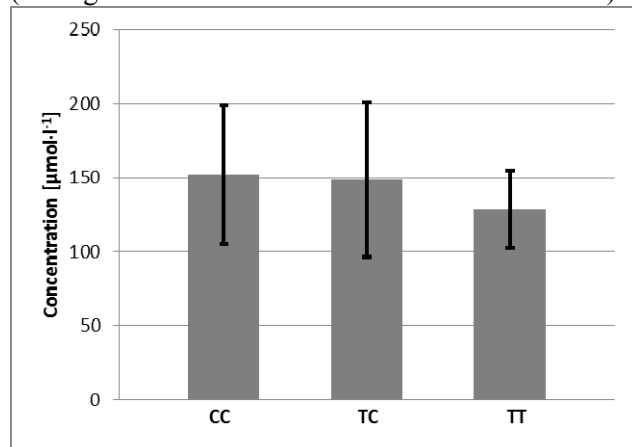
Fig. 2 Relation of genotype of Czech Pied bulls for leptin gene and level of urea in blood serum (average columns with one standard deviation bars).



Animals with the genotype CC for SNP leptin gene showed signs of higher value of urea concentration in comparison with the other genotype groups,

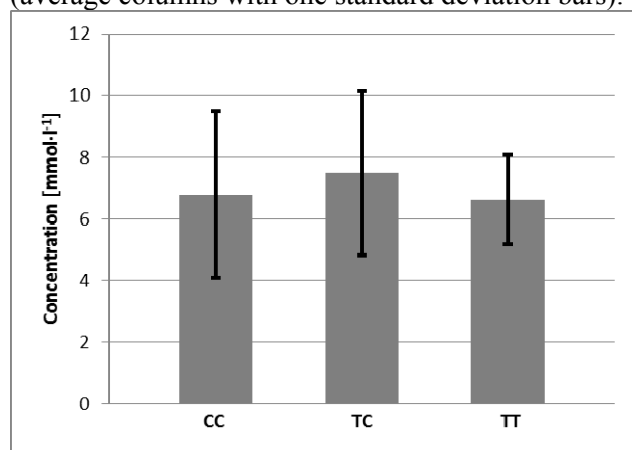
although the relation of urea to the leptin genotype was not found statistically significant. If genotype CC showed the lowest level of leptin in blood plasma, the results were in agreement with results of [6].

Fig. 3 Relation of genotype of Czech Pied bulls for leptin gene and level of creatinin in blood serum (average columns with one standard deviation bars).



Relation between creatinine and leptin genotype of Czech Pied bulls was significant. Animals with genotype TT did have the level of creatinin lower comparing to genotypes CC and TC. The study [2] focused on comparison of level of leptin and creatinin on Holstein and Charolais determined negative correlation between leptin and plasmatic creatinin.

Fig. 4 Relation of genotype of Czech Pied bulls for leptin gene and level of glucose in blood serum (average columns with one standard deviation bars).



Genotype TC had higher concentration of glucose but the influence was not significant. [9] stated that glucose did not influence leptin concentration in blood plasma of small ruminants in short horizon, even not the hormone with influence e.g. insulin. [9]

On the contrary in the case of mice insulin influenced directly leptin production by adipocytes [2]. Compared the leptin and glucose of the both the dairy and beef cattle and observed the positive correlation.

Conclusion

The effects of SNP of leptin gene were determined. In this study, we found significant effect of leptin SNP on serum creatinine concentration, but any effect of leptin SNP on other investigated indicators was noted.

Acknowledgement

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References:

- [1] Bartha T, Sayed-Ahmed A, Rudas P, Expression of leptin and its receptors in various tissues of ruminants. Domestic animal endocrinology, 2005, 29.1: 193-202.
- [2] Bellmann O, et al., Beef versus dairy cattle: a comparison of metabolically relevant hormones, enzymes, and metabolites. Livestock Production Science, 2004, 89.1: 41-54.
- [3] Buchanan FC, et al., Association of a missense mutation in the bovine leptin gene with carcass fat content and leptin mRNA levels. Genetics Selection Evolution, 2002, 34.1: 105-116.
- [4] Daix M, et al., Relationship between leptin content, metabolic hormones and fat deposition in three beef cattle breeds. The Veterinary Journal, 2008, 177.2: 273-278
- [5] Delavaud C, Ferlay A, Faulconnier Y, Bocquier F, Kann G, and Chilliard Y, Plasma leptin concentration in adult cattle: effects of breed, adiposity, feeding level, and meal intake. J Anim Sci., 2002, 80: 1317-1328.
- [6] Eryavuz A, et al. Comparison of plasma leptin, insulin and thyroid hormone concentrations and some biochemical parameters between fat-tailed and thin-tailed sheep breeds. Revue de médecine vétérinaire, 2007, 158.5: 244-249.
- [7] Houseknecht KL, et al., The biology of leptin: a review. Journal of Animal Science, 1998, 76.5: 1405-1420.
- [8] Hyder I, et al., Effect of different dietary energy levels on physio-biochemical, endocrine changes and mRNA expression

- profile of leptin in goat *Capra hircus*). *Livestock Science*, 2013, 152.1: 63-73.
- [9] Kauter K, et al., Adrenaline, insulin and glucagon do not have acute effects on plasma leptin levels in sheep: development and characterisation of an ovine leptin ELISA. *Journal of endocrinology*, 2000, 166.1: 127-135.
- [10] Keisler DH1, Daniel JA, Morrison CD, The role of leptin in nutritional status and reproductive function. *J Reprod Fertil Suppl*. 1999; 54:425-35.
- [11] Koo J, Pak K, Kim K, et al., The relationship between plasma leptin and nutritional status in chronic hemodialysis patients. *J Korean Med Sci*, 1999; 14: 546-51
- [12] Liefers SC, et al., Associations between leptin gene polymorphisms and production, live weight, energy balance, feed intake, and fertility in Holstein heifers. *Journal of Dairy Science*, 2002, 85.6: 1633-1638.
- [13] Morrison CD, Leptin resistance and the response to positive energy balance. *Physiology & behavior*, 2008, 94.5: 660-663.
- [14] Nkrumah JD, Li C, Yu J, Basarab JA, Guercio S, Meng Y, Murdoch B, Hansen C, and Moore SS, Association of a single nucleotide polymorphism in the bovine leptin gene with feed intake, growth, feed efficiency, feeding behavior and carcass merit. *J Anim Sci.*, 2004, 84: 211-219.
- [15] Yang D, et al., Association of Polymorphisms of Leptin Gene with Body Weight and Body Sizes Indexes in Chinese Indigenous Cattle. *Journal of Genetics and Genomics*, 2007, 34.5: 400-405.
- [16] Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, and Freidman JM, Positional cloning of the mouse obese gene and its human homologue. *Nature*, 1994, 372: 425-432.