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THE INFLUENCE OF SINGLE NUCLEOTIDE POLYMORPHISM IN THE LEPTIN GENE ON SERUM ENERGY METABOLISM PARAMETERS IN CATTLE

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ABSTRACT

The aim of this study was to test hypothesis that genotype TT in Czech Pied bulls, associated with higher leptinemia, will have significant effect on energy metabolism parameters in blood serum. The experiment was performed on the Czech Pied bulls in average age of 253 ± 13 days. The animals were sorted out into three groups according to the leptin genotype. The genotype TT was represented 20 animals, CT was represented 143 animals and CC was represented 127 animals. In the blood serum β-hydroxybutyrate (BHB) and non-esterified fatty acid (NEFA) were analyzed. One-way ANOVA revealed significant relation between serum concentration of NEFA and SNP genotype in leptin gene of Czech Pied bulls, but we failed to verify the hypothesis that genotype TT could affect some of monitored parameters.

Keywords: SNP leptin, Czech Pied Cattle, beta-hydroxy butyrate, non-esterified fatty acid, blood serum

INTRODUCTION

Leptin is proteinaceous hormone product of ob gene, consisting of 146 amino acids (**Daix *et al*., 2008**). It is produced mainly by adipocytes in white adipose tissue and secreted into the blood stream (**Barb *et al*., 2001**). The concentration of leptin in blood influences food intake regulation and energy metabolism (**Delavaud *et al*., 2002**) and affecting the regulation of body composition, and meat quality in mammals. Leptin also involved in fertility and immune functions (**Liefers *et al*., 2002, Tian *et al*., 2013**). The main role of leptin could be to help animals to adapt to period of undernutrition. Indeed, the rapid decrease in leptinemia in underfeed animals could be an acute signal to stimulate refeeding behavior and glucocorticoid secretion, to decrease thyroid activity, energy expenditure, insulin sensitivity and protein synthesis and to block reproduction (**Chilliard *et al*., 2005**). The leptin gene is located on bovine chromosome 4 (BTA4) (**Buchanan *et al*., 2002**) and consists of 3 exon, with exon 2 and 3 containing the protein coding region (**Tian *et al*., 2013**). Missense mutation was identified in 2 exon of the leptin gene. A cytosine to thymine transition that of encoded an amino acid change of arginine to a cysteine (**Buchanan *et al*., 2002**) The single nucleotide polymorphism in the leptin gene has been associated with intramuscular fat levels in beef **(Pannier *et al*., 2009)**. The T allele in exon 2 has been associated with fatter carcasses in various beef breeds, also the animals with TT genotype have increased in marbling score compared with CC and CT genotypes (**Nkrumah *et al*., 2005; Pannier *et al*., 2009**). Plasma leptin levels in cattle and sheep increase linearly with increased body fat mass and with increased energy balance (**Delavaud *et al*., 2002; Buchanan *et al*., 2002**). Therefore, the study of leptin regulation should be of particular interest in providing greater understanding of energy and lipid metabolism in the beef cattle. The aim of this study was to test hypothesis that genotype TT in Czech Pied bulls associated with higher leptinemia will have significant effect on energy metabolism parameters.

MATERIALS AND METHODS

Animals

The experiment was performed on the Czech Pied bulls in average age of 253 ± 13 days. The animals were sorted out into three groups according to the leptin genotype. The genotype TT was represented 20 animals, CT was represented 143 animals and CC was represented 127 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 at -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 between 8:00 and 9:30 AM. Test tubes equipped with silicone separating gel and coagulation accelerator (Dispolab, Czech Republic) were used. The serum was centrifuged to 2,000 g for 10 minutes at 4° Celsius and further stored at temperature -20° Celsius.

Biochemical analysis

Serum energy metabolism parameters - β-hydroxybutyrate (BHB) and non-esterified fatty acid (NEFA) were analyzed on Konelab T20xt automatic analyser (Thermo Fisher Scientific, Finland) using reagents supplied by Randox Laboratories (Randox Laboratories, United Kingdom).

Statistical analysis

Changes in serum metabolites were analyzed by one-way ANOVA for factors leptin genotype. ANOVA was followed by post-hoc Fischer LSD test. All statistical analyses were performed by Statistica 8.0 statistical software (StatSoft Inc., Tulsa, USA). Data in graph represents mean ± SE. The overall level of statistical significance was defined as $p < 0.05$.

RESULTS AND DISCUSSION

In this study one-way ANOVA revealed significant relation between serum concentration of NEFA and SNP genotype in leptin gene of Czech Pied bulls (Figure 1). Fischer post-hoc test showed a significantly higher NEFA concentration in group of CC bulls (0,334 ± 0,017 $mmol.l^{-1}$) compared to bulls with CT (0,299 ± 0,014 $mmol.l^{-1}$) genotype ($p < 0.05$). Serum NEFA concentration of TT group was similar to group CC (0,333 ± 0,027 $mmol.l^{-1}$), but

there no significant difference was found. The highest serum concentration of BHB was analysed in group of bulls with genotype CC (0,492 ± 0,050 $mmol.l^{-1}$) compared to other group (CT – 0,411 ± 0,021 $mmol.l^{-1}$, TT – 0,432 ± 0,059 $mmol.l^{-1}$) but no significant relations between the groups were determined (Figure 2). Leptin is produced by white adipose tissue and is thought to serve as a feedback signal indicating the size of the fat stores. When fat mass enlarges leptin is synthesized and secreted by the white fat cells, thus providing information to the hypothalamus about the excessive amount of fat deposits (**Schwarz *et al.*, 1996**). **Buchanan *et al.* (2002)** found, that higher leptin mRNA expression was in the cattle homozygous for the thymine (T) allele. This substitution having major biological effect. Also higher concentration of blood leptin was associated with T allele in leptin genotype (**Liefers *et al*., 2002**). An increase in leptin expression could reflect a feedback response in compensation for reduced biological function. The authors hypothesized that the amino acid change from arginin to cystein is imparting a functional difference to the leptin molecule, or that the cysteine's presence in the A-helix of the leptin molecule may disrupt the binding of leptin to its receptor. Another explanation may be that the presence of another unpaired cysteine in the leptin molecule could destabilize the disulfide bridge found between the 2 existing cysteines (**Rock *et al*., 1996**). Circulating levels of triglycerides have been shown to decrease during leptin administration and recover after leptin removal (**Ormseth *et al.,* 1996**). Mobilization of triglycerides as a result of letpin-induced lipolysis may account for their decrease since an increase in free fatty acids was observed in animals receiving leptin. **Frühbeck *et al*. (1997)** showed that leptin stimulates lipolysis in adipocytes. **Cohen *et al*. (1996)** determined that recombinant leptin in experimental condition does not have the same potency as that produce endogenously, possibly as a result of posttranslational modification. Downregulation of leptin receptor on adipocytes may occur lowering of lipolysis intensity **Cohen *et al*. (1996).** Leptin was shown to repress acetyl-CoA carboxylase gene expression, lipid synthesis and biochemical reactions that contribute to lipid accumulation without the participation of the brain (**Frühbeck *et al*., 1997**). However, data of these authors indicate also that leptin may act on both of these processes by inhibiting lipogenesis and stimulating. Several researches have reported reduced leptin production in adipose tissue during starvation (**Grunfeld *et al*., 1996**). With regard to this information, we could expect higher concentration of serum NEFA in TT bulls due to intensive lipolysis in adipose tissue. But in our study this effect was not found according to **Delavaud *et al*. (2002)** who suggested strong negative correlation between blood leptin and NEFA levels. The fact that the highest concentration of NEFA was found in CC group may be explained by a saturation of receptors by supraphysiological concentrations of leptin and receptor downregulation as a defense mechanism of by changes in the intracellular signaling cascade. **Delavaud *et al*. (2002)** observed also a highly significant negative correlation between plasma leptin and beta-hydroxybutyrate in blood. In our study was higher BHB blood concentration determined in the group we assume the lowest leptin concentration as was mentioned above. This finding we could explain with inhibition of leptin release due to rapid action of catecholamines as a sympathetic nervous system regulation of lipolysis and gluconeogenesis (**Rayner *et al*., 1998**). Also **Frühbeck *et al*. (1997)** have shown that fat mobilization are modulated by nervous and endocrine signals mediated mainly by β-adrenergic agonist and certain hormones as a adrenal steroids with direct effects on lipids turnover and are also involved in lipolysis regulation. Results in this study are not well consistent with the authors who have dealt with similar topic in other breeds of cattle. We supposed, according to **Gregory *et al*. (1994)**, that differences in comparison with results found in other studies are given by differences in fattening capacities particular breeds. These authors found associations with the exon 2 SNP with carcass fat content at once - and shown differences in frequency of the thymine within the cattle breeds.

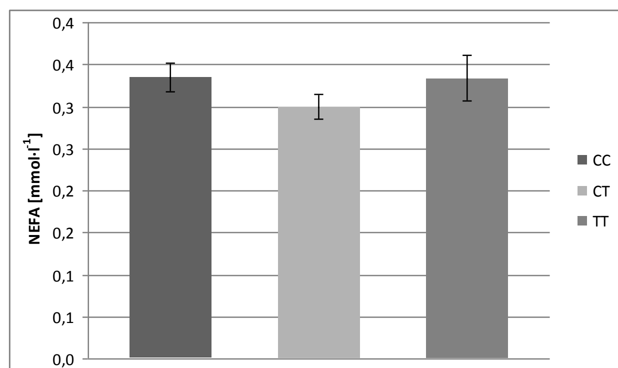

Figure 1 Relation of genotype of Czech Pied bulls for leptin gene and concentration of non-esterified fatty acid (NEFA) in blood serum. Data represent mean ± SE.

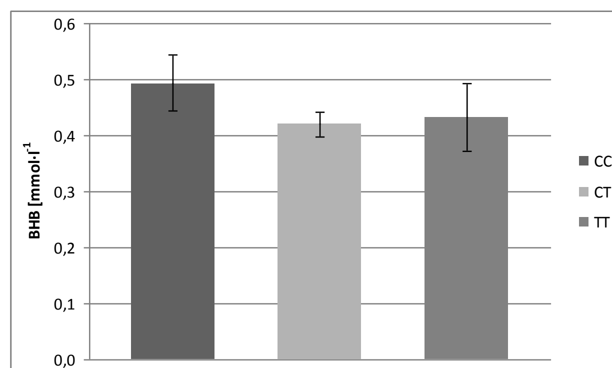

Figure 2 Relation of genotype of Czech Pied bulls for leptin gene and level of β–hydroxybutyrate (BHB) in blood serum. Data represent mean ± SE.

CONCLUSION

In conclusion, we failed to verify the hypothesis that genotype TT in Czech Pied bulls, associated with higher leptinemia, has significant effect on BHB and NEFA serum concentration. Our results suggest that changes of leptin serum concentration, which should have affect level of lipolysis and gluconeogenesis is much more regulated by the level of energy intake and by adiposity related to hypertrophy of adipose cells then by in this study monitored SNP of leptin gene.

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