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METABOLIC PARAMETERS CONCENTRATIONS IN BLOOD SERUM OF CZECH PIED BULLS DEPENDING ON SINGLE NUCLEOTIDE POLYMORPHISM OF LEPTIN GENE

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ABSTRACT

The aim of present study was to test hypothesis, that the leptin gene single nucleotide polymorphism (C/T) giving missense mutation (Arg25Cys) has an effect on concentration of blood serum total cholesterol, beta-hydroxybutyrate and urea in cattle. The experiment were performed in 58 Czech Pied bulls at 240 ± 9 days of age, which were divided in three experimental groups depending on different leptin genotypes (CC, n=28; CT, n=21; TT, n=9). Resulting genotypes in the exon 2 were CC (48.3%), CT (36.2%), and TT (15.5%). There were no differences in serum total cholesterol, urea, beta-hydroxybutyrate concentrations among the genotypes. Based on our results we may assume that analysed SNP of leptin gene have no effect on nutritional status and energy balance in fattened cattle.

Keywords: beef cattle, leptin gene, blood serum, energy balance

INTRODUCTION

Leptin is the hormone product of the obese gene synthesized and secreted predominantly by white adipocytes. This protein is supposed to be involved in the regulation of body weight by transmission of a lipostatic signal from adipocytes to the leptin receptor in hypothalamus resulting in appetite suppression and increased thermogenesis (**Zhang *et al.*, 1994**). Leptin has been implicated in several systems such as regulation of energy, metabolism, and reproduction through endocrine, paracrine, and autocrine mechanisms (**Williams *et al.*, 2002**). Leptin is sensitive to dietary manipulation and appears to play an important role in transmitting the status of energy reserves to the central nervous system to regulate feed intake and reproductive function in ruminants (**Zieba *et al.*, 2005**). The leptin gene has been mapped to bovine chromosome 4 (**Stone *et al.*, 1996**). Polymorphisms in the leptin gene have been associated with feed intake, milk yield (**Liefers *et al.*, 2002**) and body fatness (**Buchanan *et al.*, 2002; Nkrumah *et al.*, 2004**). Leptin is synthesized and released into the bloodstream in direct proportion to the amount of body fat, reflecting primarily the triacylglycerols content of lipid depots, but also functioning as a sensor of energy balance (**Chilliard *et al.*, 2005**). Circulating urea and others metabolites concentrations are good indicators of nutritional status and beta-hydroxybutyrate (BHB) as well as cholesterol are used as a indicators of energy balance in ruminants (**Bouchat *et al.*, 1981**).
The aim of present study was to test hypothesis that there is exist an effect of leptin gene single nucleotide polymorphism on some serum metabolites concentration in Czech Pied bulls.

MATERIAL AND METHODS

The experiment were performed in 58 Czech Pied bulls at 240 ± 9 days of age, which were divided in three experimental groups depending on different leptin genotypes (CC, n=28; CT, n=21; TT, n=9). There were no significant differences in age and body weight (291 ± 11 kg) among the groups. The feeding ration was based on corn silage (Table 1).

Table 1 Components and nutrients composition of the diets of 240 days old Czech Pied bulls divided into 3 groups depending on leptin single nucleotide polymorphism (TT, CT, CC).

Component/nutrient	Value
Corn silage (kg·day^{-1})	15
Clover haylage (kg·day^{-1})	5
CCM (kg·day^{-1})	1.5
Hay (kg·day^{-1})	0.5
Straw (kg·day^{-1})	0.5
Wheat meal (kg·day^{-1})	1.7
Maize meal (kg·day^{-1})	0.6
Rapeseed meal (kg·day^{-1})	0.5
Limestone powder (kg·day^{-1})	0.08
Feed salt (kg·day^{-1})	0.05
Mineral-vitamin feedstuffs for cattle (VVS, Czech Republic) (kg·day^{-1})	0.18
Crude protein (g·kg^{-1})	125.5
Crude fiber (g·kg^{-1})	173.6
Net energy (MJ·kg^{-1})	6.1
PDIE (g·kg^{-1})	80.6
PDIN (g·kg^{-1})	82.3

CCM – corn cob mix, PDIE - protein supplied when energy is limited in the rumen, PDIN - protein supplied when nitrogen is limited in the rumen

Leptin genotypes analysis

Blood samples (2 ml) were collected into tubes with EDTA stored at -20 °C. Genomic DNA was isolated from the samples using the QIAamp DNA Blood Mini Kit (Qiagen Inc., Valencia, CA, USA). The quality of DNA was verified by 1% agarose gel electrophoresis and sequential visualization with ethidium bromide. Genotypes were determined based on molecular genetic analysis of single-nucleotide polymorphism (SNP) in the exon 2 of the bovine leptin gene (transition C → T) (**Buchanan et al., 2002**). 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 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 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.

Collection of blood samples

Blood for metabolite analyses was collected randomly from *vena jugularis externa* of bulls in three groups between 8.00 and 9.30 a.m., and sampled into the test tube with silicon gel separator and coagulation accelerator (Dispolab, Czech Republic). Serum was separated by centrifugation with 2,000 x g for 10 min at 4°C, and was stored at -20 ^{o}C until analyzed.

Hormones and metabolite analyses

Total cholesterol, beta-hydroxybutyrate and urea were analysed on Konelab T20xt automatic analyser (Thermo Fisher Scientific, Finland) using currently available commercial kits (Biovendor-Laboratorni medicina, Czech Republic and Randox Laboratories, UK).

Statistical evaluation

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 represent mean. The overall level of statistical significance was defined as $p < 0.05$.

RESULTS AND DISCUSSION

In this study we determined concentrations of total cholesterol, beta-hydroxybutyrate and urea in blood serum of fattened Czech Pied bulls. There were not significant differences among the experimental group (Fig 1-3). **Fruhbeck *et al.* (1997)** in vitro and **Siegrist-Kaiser *et al.* (1997)** in vivo have demonstrated leptin effect on increasing lipolytic rates of the adipocytes. Also **Chilliard *et al.* (2005)** have shown effects on adipose tissue lipolysis. **Buchanan *et al.* (2002)** as well as **Liefers *et al.* (2002)** found, that T allele was associated with higher concentration of blood leptin. We would expected, that intensity of lipolysis as well as serum lipids concentrations could be higher in TT bulls compared to other experimental groups. Nevertheless, no differences in serum total cholesterol were found in this experiment (Fig 1).
Subsequently, there are not studies describing relationship of leptin, BHB and urea concentrations in fattened bulls. But generally, leptin acts on a receptor localized in the hypothalamus, and elsewhere in brain, to regulate energy balance and other systems (**Tartaglia *et al.*, 1995**). **Accorsi *et al.* (2005)** suggest that, in dairy cows, leptin may represent a metabolic signal of animal's status of fattening and nutritional level. **Dubuc *et al.* (1998)** found positive correlation of leptin and BHB in men.

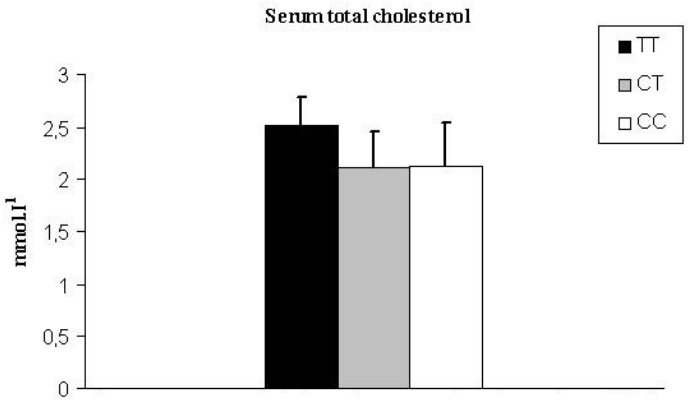

Figure 1 Serum total cholesterol concentration of 240 days old bulls divided into 3 groups depending on leptin single nucleotide polymorphism (TT, CT, CC)

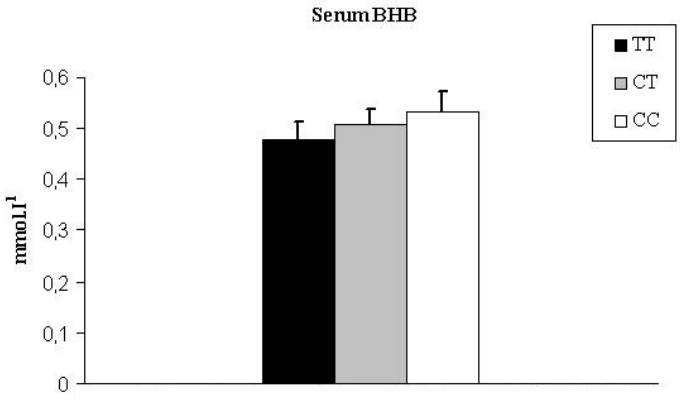

Figure 2 Serum BHB concentration of 240 days old bulls divided into 3 groups depending on leptin single nucleotide polymorphism (TT, CT, CC)

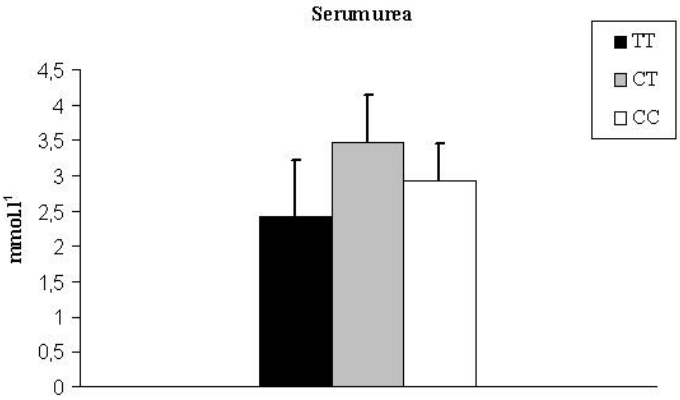

Figure 3 Serum urea concentration of 240 days old bulls divided into 3 groups depending on leptin single nucleotide polymorphism (TT, CT, CC)

CONCLUSION

In this study, there were investigated the effects of single nucleotide polymorphism of leptin gene on blood serum concentrations of chosen metabolic parameters in Czech Pied bulls. We found not significant effect of leptin SNP on serum total cholesterol, urea and beta-hydroxybutyrate concentrations.

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