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ScienceDirect
Domestic Animal Endocrinology 37 (2009) 243–249
DOMESTIC ANIMAL ENDOCRINOLOGY
www.domesticanimalendo.com

Plasma hormone and metabolite concentrations involved in the somatotropic axis of Japanese Black heifers in association with growth hormone gene polymorphism

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Received 24 March 2009; received in revised form 14 July 2009; accepted 15 July 2009

Abstract

Bovine growth hormone (bGH) gene polymorphism of leucine (Leu)-threonine (Thr) (allele A), valine (Val)-Thr (allele B), and Val-methionine (Met) (allele C) at codons 127 and 172 was shown to relate with carcass trait variations in Japanese Black cattle. In this study, 10-mo-old Japanese Black heifers with growth hormone (GH) genotypes AA, AB, BB, AC, BC, and CC (N = 141) were compared for basal GH, insulin-like growth factor-1 (IGF-1), insulin, ghrelin, glucose, and nonesterified fatty acid (NEFA) concentrations. Growth hormone release was also measured as response to growth hormone–releasing hormone (GHRH) (0.4 μg/kg body weight [BW]) using 18 heifers with GH genotypes AA, BB, and CC (n = 6 for each group). The genotype AA heifers showed the greatest BW among genotypes ($P < 0.05$). Genotype AC, BC, and CC heifers showed greater GH concentrations than genotype AA, AB, or BB heifers, in which genotype CC heifers had the highest concentrations ($P < 0.05$). However, IGF-1 concentrations did not significantly differ. The genotype AA and BB heifers had a greater GH release at 60 min following GHRH injection than did the genotype CC heifers. The area under the curve (AUC; $P < 0.07$) and incremental area (IA; $P < 0.08$) of GH responses to the GHRH challenge tended to be the highest in the genotype AA heifers and the lowest in the genotype CC heifers. In conclusion, GH gene polymorphism altered GH, which may have contributed to differences in BW and carcass traits among genotypes.

Keywords: GH genotype; Japanese Black; Heifers; Hormones; Metabolites

1. Introduction

Bovine growth hormone (bGH) is a protein hormone of 190/191 amino acids translated by the GH gene located in chromosome 19 and secreted from somatotrophs in the anterior pituitary gland under the regulation of growth hormone–releasing hormone (GHRH) and somatostatin (SS) [1]. Its secretion is also

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doi:10.1016/j.domaniend.2009.07.002

stimulated by ghrelin released from the stomach of primates and ruminants [2,3]. Growth hormone is widely known to exert some of its effects indirectly by stimulating IGF-1 secretion from the liver, which has roles in promoting cell growth [4]. In adipose tissues, GH increases lipolysis of triglyceride and alters the cell sensitivity to insulin, leading to a diabetic condition. In muscle, GH promotes protein accretion and synthesis [1]. Houseknecht et al. [5] showed that GH attenuated the ability of insulin to stimulate leptin expression in bovine adipose tissues in vitro, whereas GH treatment up-regulated adipose tissue leptin expression in vivo. Hence, the GH gene could be useful as a genetic marker to screen superior animals owing to its importance for animal growth and production.

The single-nucleotide polymorphism (SNP) observed in exon 5 of the bGH gene caused the substitution of leucine (Leu, CTG) to valine (Val, GTG) and threonine (Thr, ATG) to methionine (Met, ACG) at codons 127 and 172 of GH, respectively. Consequently, there are 3 haplotypes at these codons, namely, Leu-Thr, called allele A; Val-Thr, called allele B; and Val-Met, called allele C. Thus, there are 6 possible combinations of genotypes, namely, AA, AB, BB, AC, BC, and CC [6]. Distribution of GH alleles A and B was found to be varied among breeds and regions, in which allele A had a relatively greater frequency than allele B [7–9]. However, until now, allele C has been found only in Japanese domestic cattle, called Japanese Black and Japanese Brown cattle, and the allele distribution varied among regions in Japan [10–12]. Regarding the SNP effects on cattle production, it is reported that Holstein cattle with GH genotype AA had greater milk production than that of cattle with genotype AB [8,13]. Also, Japanese Black calves with genotype AA had greater body weights (BW) than those of cattle with genotype BB [14,15]. Moreover, allele A was associated with greater carcass weight (CW), whereas allele C gave greater marbling scores [12].

The SNP effects on endocrine function have also been studied in steers with genotypes AA, AB, and BB [14,15], however, female cattle and animals with genotype CC were not included. Based on results from our previous study, the SNP effects on carcass traits were more obvious in Japanese Black heifers than in steers [18], so it seems that GH gene polymorphism would act differently on endocrine functions in both sexes as well. Therefore, the aim of this study was to determine the effect of GH gene polymorphism on GH, IGF-1, insulin, glucose, and nonesterified fatty acid (NEFA) concentrations using 10-mo-old Japanese Black heifers.

Table 1
Ingredients and composition of feed given to Japanese Black heifers (amount fed per head daily)

Concentrate (kg DM)	1.8
Roughage (kg DM)	3.7
Total (kg DM)	5.5
Total digestible nutrients (%)	63.6
Crude protein (%)	13.2
Neutral detergent fiber (%)	51.5
Ether extract (%)	3.3
Nonfiber carbohydrate (%)	25.9

Abbreviation: DM, dry matter.

2. Material and methods

2.1. Animals

We determined GH genotypes in 158 Japanese Black heifers at Motobu farm, Okinawa prefecture, Japan. Heifers with genotypes AA, BB, or CC were selected for measurement of endocrine function. Heifers were housed in separate stalls and fed a total mixed ration (TMR), as shown in Table 1. The roughage was mainly composed of timothy hay, oat hay, and wheat hay. The concentrate was a commercial fattening diet. Measurement of BW was carried out for 63 heifers with genotype AA, BB, and CC and an average age of 285 d. Animals were treated according to the "Guiding Principles for the Care and Use of Animals in the Field of Physiological Sciences" (The Physiological Society of Japan), and the present experiment was approved by the Animal Care Committee of Tohoku University.

2.2. Bovine GH genotyping

We collected 200-μL whole-blood samples from jugular veins of 158 Japanese Black heifers and subjected them to genomic DNA isolation using the QuickGene-800 automatic nucleic acid isolation system (Fujifilm, Tokyo, Japan). The eluted DNA concentration was measured using the GeneQuant DNA/RNA calculator (Amersham Pharmacia Biotech, Tokyo, Japan). The GH genotype of cattle was identified by allele-specific multiplex polymerase chain reaction (ASM-PCR) according to the method of Chikuni et al. [6], with a slight modification. Allele-specific multiplex-PCR was carried out in 15-μL reaction mixtures containing 150 ng of template DNA, 10 mM Tris HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 200 μM each of dNTPs, 0.5 units of AmpliTaq Gold DNA polymerase (Applied Biosystems, Foster City, CA, USA), 10 pmol each of common primers GH4F and GH5R, and 5 pmol