Mapping and polymorphism of bovine ghrelin gene

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Introduction

Ghrelin, a growth hormone (GH)-releasing peptide, was isolated from rat and human stomach as an endogenous ligand for the growth hormone secretagogue receptor in 1999. Ghrelin has been identified in bovine oxyntic glands of the abomasum. Rat ghrelin stimulates GH release from bovine pituitary cells in vitro and in vivo. It circulates at considerable plasma concentrations in cattle. It has been reported that plasma ghrelin levels decrease 1h after feeding and then return to the prefeeding levels in cow. This peptide may function in the regulation of feeding or energy balance in ruminants.

Aim

Given the influences of ghrelin on the growth hormone axis and the regulation of feeding, this work aims at the study of the bovine ghrelin gene in order to evaluate the potential involvement of ghrelin in genetic variation for growth performances or milk yield.

Results

Sequencing and identification of polymorphisms

Principle: Total RNA was extracted from bovine abomasum by TriPure Isolation Reagent (Roche Applied Science). Complete ghrelin mRNA was sequenced by rapid amplification of DNA ends (RACE Smart™ RACE cDNA Amplification Kit, Clontech). Complete and partial sequences of unknown introns were determined by genome walker (Universal Genome Walker Kit, Clontech).

Results: The bovine ghrelin gene contains 5 exons and 4 introns with a short noncoding first exon of 21 bp similar to mouse and human ghrelin gene. The screening for polymorphisms revealed a total of 3 SNPs.

Mapping of bovine ghrelin gene

Using a radiation hybrid panel, the gene was mapped to chromosome 22 near microsatellite markers UWA49, BMH102, BMH192, BMH613 and UWR035 with good LOD Score. Some studies detected different QTLs near these markers. Brocades et al. (2003) detected a QTL for milk fat percent near UWA49 and Ashwell et al. (2004) reported QTLs for milk protein percent and somatic cell score near BMH410.

In order to evaluate the potential involvement of ghrelin in genetic variation for milk fat percent, milk protein percent and somatic cell score, an association study between SNPs on ghrelin gene and these traits could be performed in a cattle population.

Genotyping by single-base extension (SBE) and electrophoresis

Principle: For genotyping of polymorphic sites, amplifying and extension primers were designed for single-base extension (SBE). Primer extension reactions were performed with the S NaPshot Multiplex Kit (Applied Biosystems).

Results: Table 1. Genotype and allele frequencies observed in two different bovine breeds. The Holstein group include 94 spontaneously available bulls used in Wallon region and the Belgian White Blue group include 66 bulls from a bull-fattening enterprise.

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Statistical analysis

Statistical analysis was performed using the GLM procedure of SAS. The model used was a fixed model:

\[ y = Xb + e \]

Where:

- \( y \) = vector of estimated breeding value of bulls;
- \( b \) = unknown vector of random residual effects.
- \( e \) = vector of estimated breeding value of bulls;
- \( X \) = known design matrix of fixed genotype effect, matrix linking \( y \) and \( b \);
- \( e \) = vector of random residual effects.

The genotypic coefficient represented the gene substitution effect \( \alpha \). This model was solved using the following fixed model equations:

\[ X' R X b = X' y \iff \hat{b} = (X' R X)^{-1} X' y \]

Conclusion

These results suggest that polymorphisms in bovine ghrelin gene are a promising new possibility to select for increased milk yield. However, these findings must be validated on a larger number of animals. Further genetic study is underway to investigate the ghrelin effect on performances in Belgian White Blue bulls.

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