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Genetic Diversity and Sequence Variations at Growth Hormone Loci among Composite and Hereford Populations of Beef Cattle

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ABSTRACT

A total of 194 Hereford and 235 composite breed cattle from Wokalup Research Station were used in this study. The aims of the study were to: Investigate polymorphisms in the growth hormone gene in the composite and purebred Hereford herds from the Wokalup selection experiment, compare genetic diversity in the growth hormone gene of the breeds, sequencing and compare the sequences of growth hormone loci between composite and purebred Hereford herds with published sequence from Genebank. The genomic DNA was extracted using Wizard genomic DNA purification system from Promega. Two fragments of growth hormone gene were amplified using PCR and continued with RFLP. Each genotype in both loci was sequenced. PCR products of each genotypes were cloned into PCR II, transformed, colonies selection, plasmid DNA extraction continued with cycle sequencing. Polymorphisms were found in both breeds of cattle in both loci of GH-L1 and GH-L2 of the growth hormone gene by PCR-RFLP analysis. Sequencing analysis confirmed the RFLPs data, polymorphism detected using AluI at GH-L1 is due to substitution between leusin/ valine at position 127, while polymorphism at the MspI restriction site was caused by transition of C to T at +837 position.

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Key words: PCR-RFLP, sequencing, growth hormone gene, polymorphism

INTRODUCTION

Productions of composite animals by crossbreeding have been widely applied in commercial animal, due to their many advantages over purebreeding, especially in exploiting heterosis. The theoretical and empirical effects of crossbreeding are well established, particularly in the exploitation of non-additive genetic effects to improve mean levels of performance (Gregory & Cundiff, 1980). Gregory and Cundiff (1980) suggested that composite or synthetic breeds of livestock provide an attractive alternative to continuous crossbreeding systems, since they are expected to combine ease of management with the utilization of heterosis and additive genetic differences between breeds. Many studies in beef cattle have shown that advanced generations of composite breeds retain heterosis for many quantitative production traits at about the expected theoretical level of retained heterozygosity (Gregory, Cundiff & Koch, 1991a; Gregory, Cundiff & Koch, 1991b; Gregory, Cundiff & Koch, 1991c; Gregory, Cundiff & Koch, 1991d; Gregory, Cundiff & Koch, 1992). There have been no empirical studies, however, of single gene heterozygosity in composite breeds.

Many techniques can be used to detect sequence variation in certain loci. PCR-RFLP (Prosser, 1993; Unanian *et al.*, 1994) is a widely applied technique for the detection of DNA polymorphism at fragments of up to

several kilobases (Mitchelson, Cheng & Kricka, 1997). This relatively new technique detects the availability of restriction endonuclease cleavage sites at a locus in amplified DNA fragments from target template. Base changes, insertion or deletion at the restriction site will result in different lengths of DNA fragments, which can be analyzed by UV detection when the digested fragments are run on an ethidium bromide-stained agarose gel.

DNA sequencing, a more powerful technique of measuring genetic diversity that has been innovated and automated recently, has been widely used in genetics to forensic studies. By the invention of polymerase chain reaction (PCR), the technique can be effectively and efficiently performed. This technique has enabled researcher to get a complete sequence of any organisms of plants or animals to microorganisms such as bacteria faster than non-automatic one.

The aims of the study were to:

1. Investigate polymorphisms in the growth hormone gene in the composite and purebred Hereford herds from the Wokalup selection experiment.
2. Compare genetic diversity in the growth hormone gene in the composite and purebred Hereford herds.
3. Sequence fragments of the growth hormone gene of hereford and composite cattle
4. Compare the sequence between composite and purebred Hereford herds with published sequence from Genebank.

MATERIAL AND METHODS

Cattle

A total of 194 Hereford and 235 composite breed cattle from Wokalup Research Station were used in this study.

DNA Extraction

The genomic DNA was extracted using a Proteinase K/SDS digestion followed by a phenol/ chloroform extraction procedure (D. Groth, personal communication), or the Wizard genomic DNA purification system from Promega. Both methods resulted in very good quality DNA.

PCR-RFLP Analysis

All PCR amplification reactions were performed in an Omnigene thermocycler machine. The reactions were performed in a 50 ml reaction mix consisting of 200 ng of template DNA, 0.15 μM each of the oligonucleotide primers, 200 μM each dNTPs, 2 mM $MgCl_2$, 10x buffer and 1.5 units Taq DNA polymerase (Biotech, Australia) in 0.6 μl PCR reaction tube.

PCR products were used directly in the restriction endonuclease digestion. Agarose gel electrophoresis was carried out using 1-2% of agarose (Promega) in TAE buffer (40mM Tris-HCl; 20mM Acetate; 2mM EDTA, pH adjusted to 7.9). Electrophoresis was performed using horizontal gels, in electrophoretic cells (Bio-Rad, Richmond, U.S.A). Ethidium bromide was included in the gel at a final concentration of 0.5ug/ml (Sambrook *et al.*, 1989). After electrophoresis, DNA was visualized under UV-illumination and photographed using Polaroid type 57 film with a red filter.

Cloning PCR Products for Sequencing

The methods for producing PCR products for cloning, cloning into PCR II, transformation, blue white selection of colonies, PCR selection, plasmid DNA extraction from liquid culture, cycle sequencing, and purification of extension products are as described by Sutarno (1998)

Cycle Sequencing

Representative growth hormone genotypes were sequenced using a dye-labeled terminator cycle sequencing kit supplied by Applied Biosystems. The performance of the kit relies on the terminator premix contain A-, C-, G- and T-Dye Terminator, dITP, dATP, dCTP, dTTP, Tris-HCl (pH 9.0), MgCl2, thermal stable pyrophosphatase, and AmpliTaq DNA Polymerase, FS (Applied Biosystems). In enzymatic sequencing, a dye label is incorporated into the DNA along with the terminating base.

Aliquots of 5 μl of PCR products (growth hormone gene) or 2 μl of double stranded DNA extracted from the cloned vector containing an insert of the growth hormone gene, were added into a microcentrifuge tube consisting of 8.0 ul terminator ready reaction

mix, 3.2 pmole primer and dH_2O adjusted to final volume of 20 µl followed by cycle sequencing reaction.

The cycle sequencing reaction was performed in a thermal cycler as follows: 1). Twenty five cycles of rapid thermal ramp to 96°C, 96°C for 30 seconds, rapid thermal ramp to 50°C, 50°C for 15 seconds, rapid thermal ramp to 60°C, 60°C for 4 minutes; 2). Rapid thermal ramp to 4°C and hold.

Purification of Extension Products

Extension products were purified using the QIAquick PCR Purification Kit (Qiagen, Germany). The procedures are as described by manufacturer. The product was then stored at -20°C prior to sequencing.

Data Analysis

Genetic diversity was described by the mean number of alleles per locus (A), mean observed heterozygosity (H_o) and mean expected unbiased heterozygosity (H_e; Nei 1978), with variance calculated by the method of Nei (1978). For each locus, genotypic frequencies expected under Hardy Weinberg equilibrium were calculated from allelic frequencies using Levene's correction (Levene, 1949) for small sample size. Deviation of observed from expected frequencies were tested by X^2, and the extent of deviation expressed by Wright's fixation index, with the approximate variance of Brown (1970). Associations between the loci were examined with Burrow's composite measure of linkage disequilibrium ($_{AB}$), tested for significance by X^2 as outlined by Weir (1990)

CLUSTAL W (1.4) multiple sequence alignment was used to compare sequence between genotype in both loci of GH-L1 and GH-L2 and sequence published in Genebank.

RESULTS

Genetic Diversity

Genetic diversity at the growth hormone loci of composite and Hereford cattle are shown in Table 1. Differences in expected heterozygosity and nucleotide diversity between breeds were not significant. There were no differences in genetic diversity between sexes in either breed (data not shown).

Table 1. Standard measures of genetic diversity (± standard error) over both loci of the growth hormone gene for composite and Hereford cattle.

Breed	N	Observed heterozygosity	No of alleles	Unbiased expected heterozigosity
Composite	211	.371 ± .275	2.00 ± .000	.425 ± .060
Hereford	165	.256 ± .131	2.00 ± .000	.261 ± .143

Allelic Frequencies

Allelic frequencies at the growth hormone loci in composite and Hereford breeds are shown in Table 2. There were no significant differences in allelic frequencies between sexes in either breed for any locus (data not shown).

Table 2. Allelic frequencies at both loci of the growth hormone gene for composite and Hereford breeds.

Breed	N	GH-L1		GH-L2	
		L	V	MspI (+)	Msp I (-)
Composite	211	.589	.411	.757	.243
Hereford	165	.765	.235	.915	.085

Linkage Disequilibrium

There were significant associations between the two growth hormone loci in both breeds (Table 3).

Table 3. Estimates of Burrows composite measure of linkage disequilibrium ($_{AB}$ ± SE) for the two growth hormone loci in composite and Hereford breeds of cattle.

Breed	$_{AB}$	P
Composite	13.68 + 0.161	<0.001
Hereford	5.39 + 0.008	<0.05

Sequence comparison between genotypes (SUT-GH-LL, VV and LV) of Growth hormone gene locus 1 (GH-L1) and sequence published in Genebank were shown above. The similarities and differences were shown with or without an asterisk sign. While sequence comparisons between genotypes (SUT-GH-LL, VV and LV) of Growth hormone gene locus 2 (GH-L2) and sequence published in Genebank were shown below. The similarities and differences were shown with or without an asterisk sign.

CLUSTAL W (1.4) multiple sequence alignment.

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GHL1-GENEBANK   GCTGCTCCTGAGGGCCCTTCGGCCTCTCTGTCTCTCCCTCCCTTGGCAGGAGCTGGAAGA
SUT-GH2-LL      GCTGCTCCTGAGGGCCCTTCGGCCTCTCTGTCTCTCCCTCCCTTGGCAGGAGCTGGAAGA
SUT-GH1-VV      GCTGCTCCTGAGGGCCCTTCGGCCTCTCTGTCTCTCCCTCCCTTGGCAGGAGGTGGAAGA
SUT-GH3-LV      GCTGCTCCTGAGGGCCCTTCGGCCTCTCTGTCTCTCCCTCCCTTGGCAGGAGGTGGAAGA
                **************************************************** *******

GHL1-GENEBANK   TGGCACCCCCCGGGCTGGGCAGATCCTCAAGCAGACCTATGACAAATTTGACACAAACAT
SUT-GH2-LL      TGGCACCCCCCGGGCTGGGCAGATCCTCAAGCAGACCTATGACAAATTTGACACAAACAT
SUT-GH1-VV      TGGCACCCCCCGGGCTGGGCAGATCCTCAAGCAGACCTATGACAAATTTGACACAAACAT
SUT-GH3-LV      GGCACCCCCCCGGGCTGGGCAGATCCTCAAGCAGACCTATGACAAATTTGACACAAACAT
                 *   *******************************************************

GHL1-GENEBANK   GCGCAGTGACGACGCGCTGCTCAAGAACTACGGTCTGCTCTCCTGCTTCCGGAAGGACCT
SUT-GH2-LL      GCGCAGTGACGACGCGCTGCTCAAGAACTACGGTCTGCTCTCCTGCTTCCGGAAGGACCT
SUT-GH1-VV      GCGCAGTGACGACGCGCTGCTCAAGAACTACGGTCTGCTCTCCTGCTTCCGGAAGGACCT
SUT-GH3-LV      GCGCAGTGACGACGCGCTGCTCAAGAACTACGGTCTGCTCTCCTGCTTCCGGAAGGACCT
                ************************************************************

GHL1-GENEBANK   GCATAAGACGGAGACGTACCTGAGGGTCATGAAGTGCCGCCGC
SUT-GH2-LL      GCATAAGACGGAGACGTACCTGAGGGTCATGAAGTGCCGCCGC
SUT-GH1-VV      GCATAAGACGGAGACGTACCTGAGGGTCATGAAGTGCCGCCGC
SUT-GH3-LV      GCATAAGACGGAGACGTTCCTGAGGGTCATGAAGTGCCGCCGC
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CLUSTAL W (1.4) multiple sequence alignment.

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GHL2-GENEBANK   CCCACGGGCAAGAATGAGGCCCAGCAGAAATCAGTGAGTGGCAACCTCGGACCGAGGAGC
SUT-GH4-        CCCACGGGCAAGAATGAGGCCCAGCAGAAATCAGTGAGTGGCAACCTCGGACCGAGGAGC
SUT-GH6+-       CCCACGGGCAAGAATGAGGCCCAGCAGAAATCAGTGAATGGCAACCTCGGACCGAGGAGC
SUT-GH5++       CCCACGGGCAAGAATGAGGCCCAGCAGAAATCAGTGAATGGCAACCTCGGACCGAGGAGC
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GHL2-GENEBANK   AGGGGACCTCCTTCATCCTAAGTAGGCTGCCCCAGCTCTCCGCACCGGGCCTGGGGCGGC
SUT-GH4-        AGGGGACCTCCTTCATCCTAAGTAGGCTGCCCCAGCTCTCCGCACTGGGCCTGGGGCGGC
SUT-GH6+-       AGGGGACCTCCTTCATCCTAAGTAGGCTGCCCCAGCTCTCCGCACTGGGCCTGGGGCGGC
SUT-GH5++       AGGGGACCTCCTTCATCCTAAGTAAGCTGCCCCAGCTCTCCGCACCGGGCCTGGGGCGGC
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GHL2-GENEBANK   CTTCTCCCCGAGGTGGCGGAGGTTGTTGGATGGCAGTGGAGGATGATGGTGGGCGGTGGT
SUT-GH4-        CTTCTCCCCGAGGTGGCGGAGGTTGTTGGATGGCAGTGGAGGATGATGGTGGGCGGTGGT
SUT-GH6+-       CTTCTCCCCGAGGTGGCGGAGGTTGTTGGATGGCAGTGGAGGATGATGGTGGGCGGTGGT
SUT-GH5++       CTTCTCCCCGAGGTGGCGGAGGTTGTTGGATGGCAGTGGAGGATGATGGTGGGCGGTGGT
               ************************************************************

GHL2-GENEBANK   GGCAGGAGGTCCTCGGGCAGAGGCCGACCTTGCAGGGCTGCCCCAAGCCCGCGGCACCCA
SUT-GH4-        GGCAGGAGGTCCTCGGGCAGAGGCCGACCTTGCAGGGCTGCCCCAAGCCCGCGGCACCCA
SUT-GH6+-       GGCAGGAGGTCCTCGGGCAGAGGCCGACCTTGCAGGGCTGCCCCAAGCCCGCGGCACCCA
SUT-GH5++       GGCAGGAGGTCCTCGGGCAGAGGCCGACCTTGCAGGGCTGCCCCAAGCCCGCGGCACCCA
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GHL2-GENEBANK   CCGACCACCCATCTGCCAGCAGGACTTGGAGCTGCTTCGCATCTCACTGCTCCTCATCCA
SUT-GH4-        CCGACCACCCATCTGCCAGCAGGACTTGGAGCTGCTTCGCATCTCACTGCTCCTCATCCA
SUT-GH6+-       CCGACCACCCATCTGCCAGCAGGACTTGGAGCTGCTTCGCATCTCACTGCTCCTCATCCA
SUT-GH5++       CCGACCACCCATCTGCCAGCAGGACTTGGAGCTGCTTCGCATCTCACTGCTCCTCATCCA
                ************************************************************

GHL2-GENEBANK   GTCGTGGCTTGGGCCCCTGCAGTTCCTCA
SUT-GH4-        GTCGTGGCTTGGGCCCCTGCAGTTCCTCA
SUT-GH6+-       GTCGTGGCTTGGGCCCCTGCAGTTCCTCA
SUT-GH5++       GTCGTGGCTTGGGCCCCTGCAGTTCCTCA
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DISCUSSION

Polymorphisms were found in both breeds of cattle in both loci of GH-L1 and GH-L2 of the growth hormone gene. The polymorphisms were detected by PCR-RFLP and confirmed with sequence data. The variation in the loci indicated the genetic diversity between both breeds of composite synthesis and purebreed Hereford cattle. Genetic diversity was existed within and between breeds of those cattle. This finding indicated that even in one breed of cattle, variations are existed.

Further analysis using sequencing technique, confirmed the RFLPs data. Polymorphism detected using restriction enzyme AluI at growth hormone locus 1 is due to substitution between Leusin/ valine at position 127, while polymorphism at the MspI restriction site was caused by transition of C to T at +837 position as indicated in Clustal aligning of sequence analysis. It is therefore possible that the existence of polymorphisms at growth hormone loci can be used as the basis for selection related to the growth of the animals, since the gene codes for the production of growth hormone that affects the growth.

In relation with the selection, traditional methods of livestock selection based on choosing breeding stock from their performance characteristics, have contributed enormously to improving livestock production, but some limitations have still not been overcome. Schwerin *et al.* (1995) suggested that the limitations of traditional methods are most noticeable when the traits to be improved are difficult to measure or have a low heritability. Furthermore, traditional methods of selection are not very effective in the simultaneous improvement of several traits with genetically negative correlation, for example milk yield and fat percentage.

The limitations of traditional methods of livestock selection arise from the fact that most traits of economic importance are polygenic and therefore influenced by a variety of environmental and developmental factors (Schwerin *et al.*, 1995; Soller, 1994), and also that knowledge about the genes responsible for production traits, whether directly affecting or linked to a trait of importance, is still limited. It is therefore not generally possible to determine the genotype of any particular individual animal with respect to economic traits by examination of the phenotype alone (Beckmann & Soller, 1987).

Molecular genetics, which is currently undergoing an expansion in knowledge, may also make a major impact on animal breeding in general, and especially on cattle breeding. New technologies in molecular genetics potentially enable direct analysis of traits at the level of the gene. Coupled with innovations in quantitative genetics, advances in molecular genetics enable us to identify, map and measure the effects of quantitative trait loci (QTL) affecting production traits. The availability of methods for the identification of genetic variation, such as restriction fragment length polymorphism (RFLP) and minisatellite analysis, enable the construction of genetic maps for livestock species. These techniques have been enhanced by the polymerase chain reaction (PCR), which has had a revolutionary impact on molecular biological research, with applications to medical, agricultural and forensic science. PCR is a method for primer-directed enzymatic amplification of specific DNA sequences *in vitro* (Saiki *et al.*, 1988). It is capable of synthesizing millions of copies of specific DNA sequences in a simple, rapid and automated reaction (Erlich, 1989).

As indicated in the results, variations are detected by PCR-RFLP and sequencing. The availability of the techniques and many others has lead the preference of selection based on the level of DNA rather than phenotypic characteristic. Currently, such selection called marker assisted selection (MAS) is developed rapidly. This type of selection is much more accurate than traditional selection since selection can be done directly in the level of gene rather than the product of the gene.

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