ANALYSIS OF GENOTYPIC FREQUENCY OF BOVINE GROWTH HORMONE VARIANTS IN AN AUSTRALIAN COMMERCIAL HOLSTEIN-FRIESIAN DAIRY HERD

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The use of recombinant forms of growth hormone (GH) to boost milk production by up to 30% (Bauman 1992) has provided a graphic demonstration of the importance of this anabolic protein for galactopoiesis. The presence of any polymorphisms in the gene encoding this protein which may be associated with superior lactational performance, may provide a means with which to exploit this gene in quantitative selection programs to boost milk production. The use of site-specific restriction enzymes together with the power for sequence amplification through the use of the polymerase chain reaction has provided us with methods for the detection and characterisation of these polymorphisms.

Of the reports of bovine GH variants, the presence of a single base substitution (Cytosine to Guanine) in the second codon of exon 5 of the gene resulting in the expression of valine instead of leucine at position 127 in the GH sequence has provided the most promising lead for boosting lactational performance using this approach. (Lucy *et al*. 1994) The substitution is detectable with the restriction enzyme Alu-1 and may alter the biological activity of the translated protein, thereby suppressing milk synthesis. Only preliminary data have been reported in the Australian Holstein-Friesian herd.

We have genotyped a herd of Holstein Friesian dairy cattle for this mutation at the University of Sydney. DNA was isolated from whole blood using a phenol chloroform extraction procedure. From this a 461 base pair (bp) fragment was amplified by PCR. Each 20 mL reaction volume contained 100µM nucleotides, 20mM (NH4)2SO4, 75mM Tris-HCl pH 9.0, 0.1% (w/v) Tween, 4mM MgCl2, 2.5 units of Taq polymerase, 10µm of each primer (upper 5' CTAGCAGTCCAGCTTGACC 3' and lower 5' GCCATGCAATTTCCTCATT 3'), and 1µg of genomic DNA. The PCR cycle incorporated an initial denaturing step at 94°C for 5 minutes followed by 94°C for 5 seconds, 65°C for 5 seconds and 72°C for 30 seconds, for two cycles. A decrease in annealing temperature of 1°C every second cycle was continued until it reached 57°C, when 10 cycles were completed followed by a 2 minute extension at 72°C. Overnight incubation of the product with the restriction endonuclease Alu-I at 37°C yielded several fragments depending on the genotype. The leucine homozygote yielded 4 fragments of 185, 114, 111 and 51 bp, the valine homozygote yielded 3 fragments of 236, 114 and 111 bp, and the leucine/valine heterozygote yielded 5 fragments of 236, 185, 114, 111 and 51 bp. The genotypic frequencies as they relate to milk production are presented in Table 1.

Table 1. Genotypic frequency and milk production of bovine growth hormone variants in a University of Sydney dairy herd

Genotype	Number of cows	Frequency	305 day milk yield (L) mean ± s.e.m.
Leu127/Leu127	58	0.80	6030 ± 1121
Leu127/Val127	11	0.15	6060 ± 1127
Val127/Val127	4	0.05	5834 ± 760
Total	73	1.00	

The dominant genotype within the herd was the Leu^{127} homozygote with proportionately lower frequencies for the Leu/Val and Val/Val variants. Expression of the Val/Val variant was associated with lower milk production, while there was no difference between the other two genotypes. The low frequency of the Val/Val variant within the herd most likely reflects the persistent selection of animals for milk yield and therefore an inadvertent selection against this genotype. Thus the use of this polymorphism in commercial selection programs may be of little value to the dairy industry.

BAUMAN, D.E. (1992). *J. Dairy Sci.* **75**, 3432-51.

LUCY, M.C., HAUSER S.D., EPPARD, F.J., KRIVI, J.J., CLARK, J.H., BAUMAN, D.E. and COLLIER, R.J. (1993). *Dom. Anim. Endocrinol.* **10**, 325-33.