Characteristics of bovine milk protein for young dairy bulls in Korea.

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SUMMARY

This study was undertaken for genetic polymorphisms of bovine milk proteins for 17 randomly selected young bulls which were produced with a view to progeny test in Korea. Semen samples form young bulls were collected and analyzed by PCR-RFLP procedures. Genetic effects of milk proteins on production traits were also studied using daughter records from young bulls. The results obtained were as follows : 1. Amplified products of 310bp(α_{s1}-casein), 396bp(α_{s2}-casein), 498bp(β-casein), 874bp(κ-casein), 583bp(α-lactalbumin), and 961bp (β-lactoglobulin) were observed using the PCR procedure. 2. Amplified products were digested with Mae III(α_{s1}-casein), Mnl I(α_{s1}-casein), Hind III(κ-casein), Msp I(α-lactalbumin), and Hph I (β-lactoglobulin). 3. After digestion, α_{s1}-casein was detected by 214bp and 96bp for BB genotype, while α_{s2}-casein was shown by 272bp and 124bp for AA genotype. Both κ-casein and α-lactalbumin were characterized by 498bp, 259bp, 393bp, and 190bp for BB genotypes, respectively. 4. After PCR amplification with restriction enzymes, 11AA and 6AB genotypes for κ-casein, 9AB and 8BB genotypes for β-lactoglobulin, as well as 12II and 5ID genotypes for growth hormone were observed. 5. Genetic differences among genotypes (κ-casein, β-LG, bGH) for production traits studied were significant. 6. When daughter yields were grouped by AA and AB genotypes of κ-casein, means and standard deviations for all the traits studied, except milk fat percent, showed remarkable differences.

Keywords : Milk protein, Genotypic effects, Young dairy bulls

INTRODUCTION

Recent developments in molecular genetics allowed the identification of genetic markers. This tact requires that the contributions of such loci to production traits be determined. The identification of quantitative trait loci(QTL) for production traits in dairy cattle was carried out by using field data in many studies (Dentine et al., 1990 ; Weller et al., 1990).

The objectives of this study were to detect genetic polymorphisms of bovine milk proteins for 17 randomly chosen young bulls which were produced with a view to progeny test in Korea. Genotypic effects of milk proteins on production traits were also studied using daughter records from young bulls.

MATERIALS AND METHODS

1) For the isolation of DNA from bulls, semen straws were obtained from 17 randomly chosen young bulls which were progeny tested. Extraction of genomic DNAs from frozen semen was done by both Medrano et al.(1990) and Lee et al.(1995a) procedures.

Table 1. Oligonucleotide sequences and references of bovine milk protein and growth hormone loci specific DNA primer

Loci	Primer
κ-casein	5' - GTG CTG AG(T/C) AGG TAT CCT AG - 3'
	5' - GTA GAG TGC AAC AAC ACT GG - 3'
β-lactoglobulin	5' - ACC TGG AGA TCC TGC TGC AGA AAT G - 3'
	5' - CAT CGA TCT TGA ACA CCG CAG GGA T - 3'
Growth Hormone	5' - AGA ATG AGG CCC AGC AGA AAT C - 3'
	5' - GTC GTC ACT GCG CAT GTT TG - 3'

PCR was used to amplify κ-casein, β-lactoglobulin, and bovine growth hormone loci using necessary primers (Table 1). In order to amplify loci, PCR reaction mixture, annealing temperature and time were shown in Table 2.

Table 2. PCR reaction mixture, conditions and amplified fragment size(bp) of milk protein and GH loci

Loci	Primer conc.	Annealing		Fragment size (bp)
		Temperature(°C)	Time(Sec)	
κ-casein	50 pmole	57	120	874
β-lactoglobulin	60 pmole	61	120	961
Growth Hormone	50 pmole	58	80	768

2) Statistical model

$$Y = Xb + Qg + Za + e$$

where,

Y = PTA Values for milk, milk fat, milk protein, and SNF yields of 17 young bulls; **b** = a scalar unknown constant; **a** = a vector of random effects; **e** = a vector of random residuals; **X, Q, Z** = known incidence matrices corresponding to respective effects

RESULTS AND DISCUSSION

Table 3 presents the frequencies of κ-casein, β-lactoglobulin, and growth hormone genotypes and fragment size.

Table 3. Frequencies of genotype and size of restricted fragments for each locus

Loci	Genotypes	Frequencies	Fragment size(bp)
	AB	11	874
κ-casein	AB	6	874, 524, 350
	AB	9	220, 166
β-lactoglobulin	BB	8	166
	II	12	612
Growth Hormone	ID	5	705, 612

Both κ-casein and β-lactoglobulin loci were reported to effect on milk protein (Pinder et al., 1991 ; Wilkins and Kuys, 1992). On the other hand, growth hormene loci can be utilized to distinguish between higher group and lower group of milk fat contents(HØj et al., 1993).

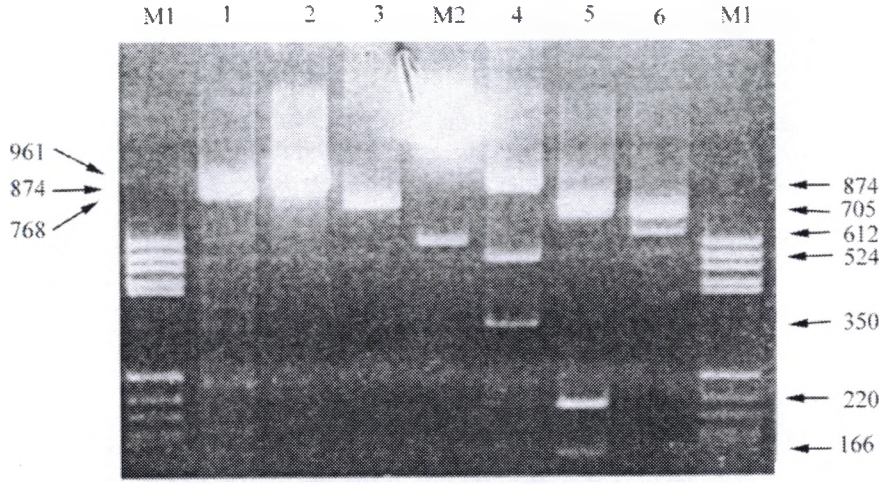

Fig. 1. Gel demonstrating the polymorphism detected by PCR.

Lane 1, 2, 3: PCR amplification(undigestion), Lane 4, 5, 6: enzyme digestion. Lane 1, 4: κ-casein, Lane 2, 5 : β-lactoglobulin, Lane 3, 6 : growth hormone, M1 : pBR322-Hae III size marker, M2 : λ DNA-HindIII size marker. Lane 1, 2, 3 showed amplified PCR products and lane 4, 5, 6 presented fragments with enzyme digestion. M1 and M2 represented size markers. PTA values for production traits and growth hormone of young bulls were shown in Table 4. Theoretically, an animal model is adapted to estimation of the contributions of single genotypes to production traits. However, the indirect analysis of sire PTA is a simple and attractive alternative to identify contributions of single genes to production traits(Cowan et al., 1992).

Table 4. PTA values for production traits and genotypes for milk protein and growth hormone of young bulls

Young bulls	Loci			PTA values						
	K-CN	B-LG	bGH	Milk Yield	Fat Yield	Fat Per.(%)	Protein Yield	Protein Per.(%)	SNF	SNF Per.(%)
120300010990	AB	BB	ID	-29.735	5.7610	0.09589	-2.19170	-0.03128	-2.9360	-0.00903
120300011016	AB	AB	II	349.062	12.2751	0.01349	2.45445	-0.12783	5.8794	-0.09738
120300011035	AA	AB	II	329.149	12.9164	0.02229	0.67395	-0.01423	1.9952	-0.06838
120300011037	AA	BB	II	324.989	17.0535	0.07199	2.83770	0.02542	1.4273	0.01827
120300011039	AB	AB	II	123.122	2.0114	0.00914	1.91685	0.01212	2.9672	-0.07868
120300011049	AB	AB	II	355.751	14.2081	0.01144	-0.51370	0.06397	-4.7477	0.02312
120300011060	AA	AB	II	83.549	12.0740	0.17684	0.25185	0.02322	3.9688	0.11287
120300011061	AA	AB	ID	184.558	3.2858	-0.03471	3.96430	0.02877	10.0121	-0.06573
120300011068	AA	AB	II	185.370	7.1447	-0.09826	-2.24470	-0.03433	-6.9819	0.15677
120300011070	AA	BB	II	448.261	9.5468	-0.09946	-0.56145	-0.04438	2.3311	-0.03298
120300011071	AB	BB	II	-50.185	4.4532	0.08884	-1.30170	0.17007	-4.9098	0.01977
120300011072	AA	AB	II	411.725	14.4939	-0.06341	4.16400	-0.00708	8.5748	0.04697
120300011073	AA	BB	II	869.144	26.6766	-0.04191	5.05175	-0.06448	17.2537	-0.02633
120300011074	AA	BB	ID	76.043	13.8014	0.21939	5.71050	0.05487	11.9012	0.01877
120300011075	AB	BB	ID	325.872	15.4086	0.07034	4.67945	0.02517	10.3342	-0.07718
120300011076	AA	BB	ID	736.164	23.4456	0.00279	2.74915	0.01152	7.4281	-0.10658
120300011077	AA	AB	II	515.297	13.9483	-0.09371	7.23535	0.13192	19.0398	0.20447

K-CN ; -casein, B-LG ; -lactoglobulin, bGH ; bovine growth hormone

Table 5 showed genetic difference for milk proteins of κ-casein, β-lactoglobulin, and growth hormone for production traits. Except for milk fat percentage, all the traits presented significant differences among genotypes within loci.
But sire PTA is a regressed statistic, and the differences among sires for single genotypes are masked by other genetic contributions. Therefore, the indirect analysis of genotypic effects on production traits using PTA values may be biased.

Table 5. Genotypic differences for milk proteins of K-CN, B-LG, and GH for production traits.

Traits	K-CN		B-LG		BGH	
	AA	AB	AB	BB	II	ID
PTA	71.80140	-134.28450	-39.53810	33.23130	29.22390	-70.13760
PTA	1.36390	-3.99370	-1.97190	2.21840	0.59560	-1.42950
PTA	-0.01315	0.02051	-0.02155	.02425	-0.01277	0.03065
PTA PRT	0.31830	-1.40450	0.13550	-0.15240	-0.33350	0.80040
PTA PRT(%)	-0.00476	0.00965	-0.00657	0.00739	0.00003	0.00008
PTA SNF	1.78340	-3.90800	0.33270	-0.37430	-1.22990	2.95180
PTA SNF(%)	0.03790	-0.03592	0.00873	-0.00983	0.00060	-0.00145

K-CN ; κ-casein, B-LG ; β-lactoglobulin, bGH ; bovine growth hormone

Table 6 showed means and standard deviations of daughters' phnotypic values according to AA and AB genotypes of κ-casein of young bulls.
Significant differences between AA and AB groups for κ-casein existed.

Table 6. Means and standard deviations of daughters' phenotypic values according to AA and AB genotypes of κ-casein locus of young bulls

Traits	K-CN				T-value (Prob)
	AA(445)*		AB(186)*		
	Mean	STD	Mean	STD	
ME MILK	8139.1995	1671.0288	7715.2393	1536.3998	2.9742(0.0031)
ME FAT	291.3336	66.6176	271.6192	60.0877	3.4863(0.0005)
ME FAT%	3.5939	0.5079	3.5319	0.4514	1.4433(0.1494)
305 PRT	221.9720	48.1187	197.9512	41.3568	3.7393(0.0002)
305 PRT%	3.3362	0.2856	3.1904	0.3415	3.4172(0.0007)
305 SNF	585.1835	121.3004	507.0978	131.6597	4.4762(0.0001)
305 SNF%	8.7863	0.4239	8.1808	1.4415	3.6081(0.0005)

* ( ) ; indicates number of daughters

REFERENCES

Cowan, C. M., M. R. Dentine, and T. Coyle. (1992) *J. Dairy sci.* **75** : 1097.
Dentine, M. R., and C. M. Cowan. (1990) *Theor. Appl. Genet.* **79** : 775.
H Øj, S., Fredholm, M., Larsen, N. J. and N. H. Nielsen. (1993) *Animal Genetics.* **24** : 91.
Lee, K. J., J. U. Kim, Y. K. Lee, M. S. Kang and S. H. Kang. (1995a) *Korean J. Anim. Sci.* **37**(3): 225-231.
Medrano, J. F., and E. Aguilar-Cordova. (1990) *Bio/Technology.* **8** : 144
Pinder, S. J., Perry, B. N., Skidmore, C. J. and D. Savva. (1991) *Animal Genetics.* **22** : 11-20.
Well, J. I., Kashi, Y. and M. Soller. (1990) *J. Dairy Sci.* **73** : 2525.
Wilkins, R. J. and Kuys, Y. M. (1992) *Animal Genetics.* **23** : 175-178.