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Identification of Polymorphisms in the Bovine Paraoxonase 1 Gene

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Abstract: The Paraoxonase 1 gene (*PON1*) encodes paraoxonase which hydrolyzes organophosphate pesticides and nerve gasses. In addition, PON1 has been shown to have significant antiatherosclerotic effects and is a major candidate gene associated with the reduction of atherosclerosis. Most of the literature reporting polymorphisms in PON1 has been in humans while less information about the polymorphism of *PON1* gene has been reported in mammals, especially in bovine species. Polymorphisms in the *PON1* gene were evaluated in Simmental x Chinese Yellow cattle (n = 32) and Longdong cattle (n = 37) using PCR-SSCP in exons 1-9 of the *PON1* gene. Results demonstrated polymorphisms in the bovine *PON1* gene at exons 4, 6, 8 and 9. DNA sequencing identified specific substitutions in exon 4, 6, 8 and 9 (g.338G>A, g.647C>T, g.896C>T and g.998T>C, respectively). Two genotypes were identified in exon 4 (GG and AG), exon 6 (TT and CT) and exon 9 (TT and CT) while 3 genotypes were identified in exon 8 (CC, CT and TT). The allelic frequency for the G allele was 92.03% and the A allele was 7.97% in exon 4. Allelic frequencies for the T and C alleles were 89.86 and 10.14%, respectively, in exon 6. In exon 8, frequencies for the C and T alleles were 94.93 and 5.07%, respectively. In exon 9, allelic frequencies for the T and C alleles were 88.41 and 11.59%, respectively.

Key words: Cattle, *PON1* gene, exon, polymorphism, PCR-SSCP, China

INTRODUCTION

The paraoxonase family of genes consists of *PON1*, *PON2* and *PON3* genes. Paraoxonase genes are located in the p7 chromosome in humans, the 6th chromosome in mice and the 11th chromosome in bovine. The Paraoxonase 1 gene (*PON1*) contains nine exons and eight introns. The length of PON1 mRNA is 1205 bp, consisting of 355 amino acids and has a relative molecular mass of 43000-45000 (Zech *et al*., 1999; Martin-Burriel *et al*., 1997; Costa *et al*., 2005). The PON1 plays a role in insulin levels, insulin resistance and high-density lipoproteins in humans (Purnell *et al*., 2003; Watzinger *et al*., 2002) and has been shown to be a major candidate gene in lipometabolism and cardiovascular disease (Aviram *et al*., 2000; Ayub *et al*., 1999). Like Lipoprotein Lipase (LPL), PON1 plays a key role in the metabolism and physiology of mammalian growth (Harel *et al*., 2004). Recently, the PON1 crystalline structure has been verified. This demonstrates that the PON1 protein has catalytic action on several substrates with the basic function of hydrolysis of ring lactones (Khersonsky and Tawfik, 2005). The PON1 protein is synergistic with insulin, steapsin, growth hormone, lipoprotein lipase and leptin (Curi *et al*., 2006a, b). The PON1 enzyme functions in the movement of lipids across cell membranes and within the cells (Purnell *et al*., 2003). Little information is available in the literature for bovine PON1. Ji *et al*. (2008a, b, 2009a, b) studied the polymorphism of exon 6 of the *PON1* gene using RFLP and analyzed associations between partial production traits and polymorphisms identified. Results showed that association of PONI/EcoR V genotypes with body weight, average daily gain, rib eye area and tenderness were evident ($p<0.05$). Variations in the *PON1* gene could be important in resilience of cattle to organophosphates and impact phenotypic expression of metabolic and physiological growth processes in cattle. Consequently,

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the objective of the study was to evaluate polymorphisms in all exons of PON1 in Simmental x Chinese Yellow cattle crosses and Longdong cattle to evaluate the potential genetic variability in this gene.

MATERIALS AND METHODS

Animals and DNA extraction: Blood samples were collected from 69 crossbred cattle from Pingliang and Kangle districts of Gansu Province representing different breed crosses: Simmental x Chinese Yellow cattle (Kangle, n = 32) and Longdong cattle (Pingliang, n = 37). Genomic DNA was extracted from the blood leukocytes using a phenol-chloroform extraction protocol followed by an ethanol precipitation step and the extraction was checked on 1% agarose gels in 1XTAE buffer.

Primer and PCR amplification: The PCR primers (Table 1) were designed from the bovine *PON1* gene nucleotide sequence of DNA in GenBank (accession number NC_007302.5, EU289337) (Ji *et al*., 2009a, b) using the primer 3 program (http://frodo.wi.mit.edu/). The PCR reaction was performed in a volume of 25 μL using genomic DNA (approximately 50-100 ng), 1 μL; 10 pmol of each primer, 1 μL; Taq DNA polymerase (5U μL^{-1}), 0.5 μL; dNTP (2.5 mM L^{-1}), 2.0 μL; 10×PCR Buffer (Mg^{2+} free), 2.5 and 17 μL ddH_2O. The PCR cycling conditions were 94°C for 3 min for the first cycle and 94°C for 30 sec for the remainder of the cycles for denaturation, 60-63.5°C (Table 1) for 30 sec for annealing and 72°C for 30 sec for polymerization; a total of 35 cycles. The final extension was at 72°C for 10 min. The amplification result was verified on 1% agarose gel (ABI 2720, USA).

Single-strand conformation polymorphism analysis: Variation in the amplicons of exon 4, 6, 8 and 9 in PON1 was screened using SSCP. The 3 μL of PCR product was mixed with 7 μL of denaturation buffer (98% deionized formamide; 10 mM EDTA pH8.0, 0.025% bromophenol blue and 0.025% xylene-cyanol). The samples were denatured at 98°C for 10 min. Samples were cooled on ice for at least 5 min and loaded onto 10% polyacrylamide gels (39:1 acrylamide:bisacrylamide). For exon 4 and exon 9, electrophoresis was carried out at 19°C and 190 V for 10 h in 1×TBE buffer; 12% polyacrylamide gels (39:1 acrylamide:bisacrylamide). For exon 6 and exon 8, electrophoresis was carried out at 19°C and 180 V for 10 h in 1×TBE buffer; gels were silver-stained (Byun *et al*., 2009).

Table 1: Primer sequences used in study

Primers	Sequence (5'-3')	Size (bp)	Annealing temperature°C
PON1-EX1F	AAGCAAGTTCCCGCAGTCC	365	63.0
PON1-EX1R	ACATCCTGCTGCCTTCTGAT		
PON1-EX2F	CAGGCAAAGTACAGTTAAGGA	323	60.6
PON1-EX2R	AAATACTCTCCTGGCTAGTAG		
PON1-EX3F	GGGAGTGATGAAGACAAG	279	63.0
PON1-EX3R	GAATGCGTACACAGGTTG		
PON1-EX4F	TGTGCTGTCTACCACCTCCAT	367	63.0
PON1-EX4R	CCCAGAGTGAGAGCATTATTC		
PON1-EX5F	TTCCATCTGCCTGACATCC	327	63.5
PON1-EX5R	CCCTGCCAAAGGAAACACT		
PON1 EX6F	ACTCTGATTTTACGAGCATAC	345	60.0
PON1 EX6R	ATGAGAATCTAGGGACAAACG		
PON1-EX7F	CCAGGCTCTAAGCAAAGT	385	62.0
PON1-EX7R	TCTGCCACTTCCCACTCTG		
PON1-EX8F	CGTATTGCTCCATCTCCTT	297	62.0
PON1-EX8R	CATCTATCCACATCTCCCT		
PON1-EX9F	AGTCTCTTCTTATGGAGAAATG	387	63.0
PON1-EX9R	AGATCTCCATCTCCTGTGATGT		

Sequencing and sequence analysis: The PCR products, two genotypes in exon 4, 6 and 9 and three genotypes in exon 8 were identified by DNA sequencing. Several inserts from positive clones for each animal were confirmed by PCR using the primers mentioned above. The SSCP patterns were determined in each positive clone and clones corresponding to different PCR-SSCP band patterns were chosen for DNA sequencing. Each distinct SSCP pattern that corresponded to different clones and individuals was sequenced (Sheng Gong Company, China). The sequence alignments, translations and comparisons were carried out with BioEdit7.0 Software (Ibis Biosciences, Carlsbad, CA, USA).

RESULTS AND DISCUSSION

The PCR amplification products of exons 4, 6, 8 and 9 of the *PON1* gene tested using SSCP and results are shown in Fig. 1-4.

Two genotypes were identified in exons 4 (GG, AG), 6 (TT and CT) and 9 (TT and CT) and 3 genotypes were identified in exon 8 (CC, CT and TT) using PCR-SSCP. Sequence alignment diagrams with the published cattle sequence (GenBank Accession EU289337) are given in Fig. 5-8. DNA sequencing identified specific substitutions

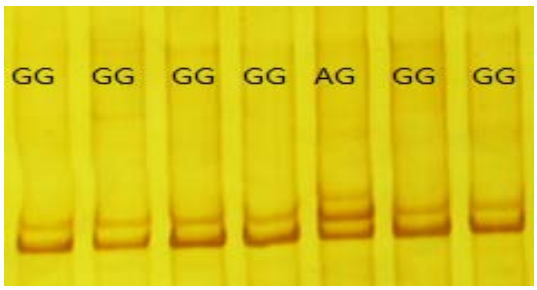

Fig. 1: *PON1* gene amplification products in SSCP at exon 4

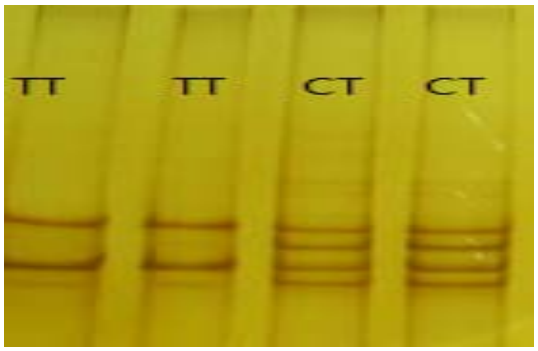

Fig. 2: *PON1* gene amplification products in SSCP at exon 6

in exon 4, 6, 8 and 9 (g.338G>A, g.647C>T, g.896C>T and g.998T>C, respectively) but without apparent alteration of amino acid (BioEdit7.0, Ibis Biosciences, Carlsbad, CA, USA). In this study, the frequencies of the two alleles in the 69 cattle studied are shown in Table 2. Allele G was the most frequent allele with a frequency of 92.03%, followed by allele A at 7.97% in exon 4. Allele T was the most frequent allele in exon 6 with a frequency of 89.86% followed by allele C at 10.14%. In exon 8, allele C was the most frequent allele with a frequency of 94.93% followed by allele T at 5.07%. In exon 9, allele T was the most frequent allele with a frequency of 88.41%, followed by allele C at 11.59% in exon 4 genotypic frequencies were 84.06% and 15.94% for GG and AG, respectively.

Fig. 3: *PON1* gene amplification products in SSCP at exon 9

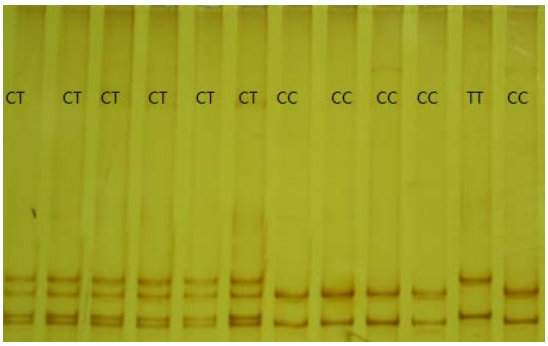

Fig. 4: *PON1* gene amplification products in SSCP at exon 8

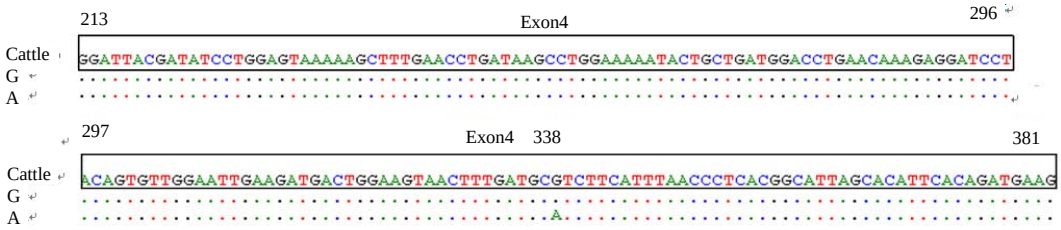

Fig. 5: Sequence alignment of the bovin exon 4 of PONI alleles together with the published cattle sequence (EU289337). Dots represent nucleotides identical to the top sequence. Nucleotides in exon 4 are shown in a solid box (Sequenced by up primer)

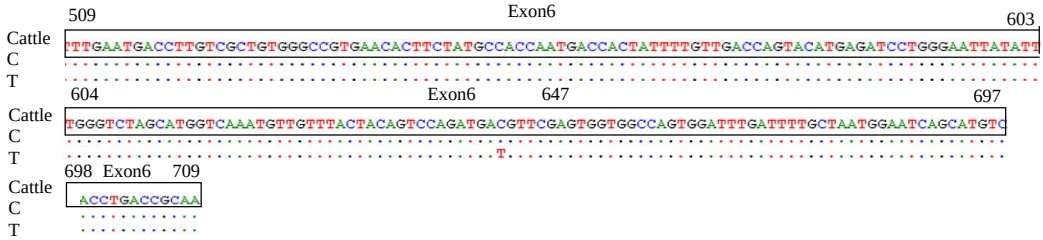

Fig. 6: Sequence alignment of the bovin exon 6 of PONI alleles together with the published cattle sequence (EU289337). Dots represent nucleotides identical to the top sequence. Nucleotides in exon 4 are shown in a solid box (Sequenced by up primer)

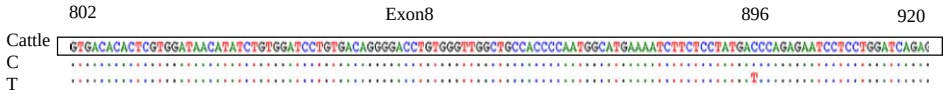

Fig. 7: Sequence alignment of the bovin exon 8 of PONI alleles together with the published cattle sequence (EU289337). Dots represent nucleotides identical to the top sequence. Nucleotides in exon 4 are shown in a solid box (Sequenced by up primer)

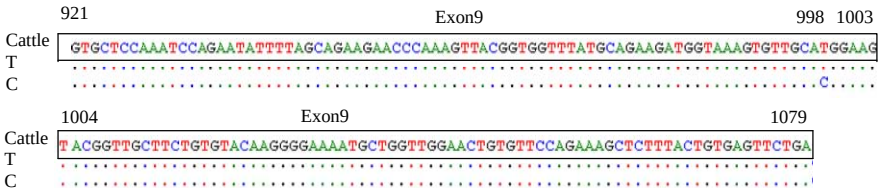

Fig. 8: Sequence alignment of the bovin exon 9 of PONI alleles together with the published cattle sequence (EU289337). Dots represent nucleotides identical to the top sequence. Nucleotides in exon 4 are shown in a solid box (Sequenced by up primer)

Table 2: Allelic and genotypic frequencies of bovine PON1

			Genotype frequency (%)		Allelic frequency (%)	
Breed population[a]	Gene	n	GG	AG	G	A
Crossed cattle1	*PONI-EX4*	32	0.8750	0.1250	0.9375	0.0625
Crossed cattle 2		37	0.8378	0.1622	0.9189	0.0811
Total		69	0.8406	0.1594	0.9203	0.0797
			Genotype frequency (%)		Allelic frequency (%)	
Breed population[a]	Gene	n	TT	CT	T	C
Crossed cattle 1	*PONI-EX6*	32	0.8125	0.1875	0.9064	0.0936
Crossed cattle 2		37	0.7838	0.2162	0.8919	0.1081
Total		69	0.7971	0.2029	0.8986	0.1014
			Genotype frequency (%)			Allelic frequency (%)	
Breed population[a]	Gene	n	CC	CT	TT	C	T
Crossed cattle 1	*PONI-EX8*	32	0.9063	0.0937	-	0.9531	0.0469
Crossed cattle 2		37	0.9189	0.0541	0.0270	0.9460	0.0540
Total		69	0.9130	0.0725	0.0145	0.9493	0.0507
			Genotype frequency (%)		Allelic frequency (%)	
Breed population[a]	Gene	n	TT	CT	T	C
Crossed cattle 1	*PONI-EX9*	32	0.8438	0.1562	0.9219	0.0781
Crossed cattle 2		37	0.7027	0.2973	0.8513	0.1487
Total		69	0.7681	0.2319	0.8841	0.1159

[a]Crossed cattle1: Simmental x Chinese Yellow cattle; [a]Crossed cattle 2: Longdong cattle

Genotypic frequencies in exon 6 were 79.71 and 20.29% for TT and CT, respectively. In exon 8, the genotypic frequency for CC was 91.30 and 7.25% for CT and 1.45% for TT. Genotypic frequencies in exon 9 were 76.81% for TT and 23.19% for CT. Researchers concluded that there are two alleles (G and A; T and C; C and T and T, C, respectively for exon 4, 6, 8 and 9) within both groups (Simmental x Chinese Yellow cattle; Longdong cattle) and G, T, C and T have the greater frequency at exons 4, 6, 8 and 9, respectively. By sequencing, researchers found polymorphisms in both breed-types, suggesting the potential for phenotypic differences resulting from gene expression. In this research, the genetic variation was detected using 69 cattle. While a greater sample size would have probably been more definitive, the current numbers were sufficient to determine that genetic differences occurred compared to the published PON1 sequence. Further research is warranted to determine more precise estimates of allelic and genotype frequencies in Chinese cattle and crosses and to determine if these polymorphisms influence production performance.

CONCLUSION

Results from this study suggest that unique polymorphisms were identified in the bovine *PON1* gene

and further study is needed to determine if they might have an impact on phenotypic expression of growth and production traits in beef cattle.

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REFERENCES

Aviram, M., E. Hardak, J. Vaya, S. Mahmood and S. Milo *et al*., 2000. Human serum paraoxonases (PON1) Q and R selectively decrease lipid peroxides in human coronary and carotid atherosclerotic lesions: PON1 esterase and peroxidase-like activities. Circulation, 101: 2510-2517.

Ayub, A., M.I. Mackness, S. Arrol, B. Mackness, J. Patel and P.N. Durrington, 1999. Serum paraoxonase after myocardial infarction. Arterioscler. Thromb. Vasc. Biol., 19: 330-335.

Byun, S.O., Q. Fang, H. Zhou and J.G.H. Hickford, 2009. An effective method for silver-staining DNA in large numbers of polyacrylamide gels. Anal. Biochem., 385: 174-175.

Costa, L.G., A. Vitalone, T.B. Cole and C.E. Furlong, 2005. Modulation of paraoxonase (PON1) activity. Biochem. Pharmacol., 69: 541-550.

Curi, R.A., D.A. Palmeiri, L. Suquiawa, A.L.J. Ferraz and H.N. de Oliveria *et al*., 2006a. Effects of *GHR* gene polymorphisms on growth and carcass traits in Zebu and crossbred beef cattle. Livestock Sci., 101: 94-100.

Curi, R.A., H.N. Oliveira, A.C. Silveira and C.R. Lopes, 2006b. Association between *IGFI*,*IGF-IR* and *GHRH* gene polymorphisms and growth and carcass traits in beef cattle. Livestock Prod. Sci., 94: 159-167.

Harel, M., A. Aharoni, L. Gaidukov, B. Brumshtein and O. Khersonsky *et al*., 2004. Structure and evolution of the serum paraoxonase family of detoxifying and anti-atherosclerotic enzymes. Nat. Struct. Mol. Biol., 11: 412-419.

Ji, A.G., S.Z. Xu, Y.H. Huai, X. Gao and Z.K. Zhou *et al*., 2008a. Polymorphisms scanning in exon of the *PON2* gene and analysis of exon9 with SSCP in seven bovine breeds. Acta Veterinaria et Zootechnica Sinica, 39: 410-416.

Ji, A.G., Y.H. Huai, Z.K. Zhou, J.Y. Li and L.P. Zhang *et al*., 2008b. Association between *PON1* gene SNPs and growth and carcass traits in beef cattle. Asian-Aust. J. Anim. Sci., 21: 1097-1102.

Ji, A.G., Z.K. Zhou, L.P. Zhang, R.J. Yang and S.Z. Xu *et al*., 2009a. *PON1* gene SNPs and association with growth and carcass traits in beef cattle. Acta Veterinaria et Zootechnica Sinica, 40: 122-128.

Ji, A.G., J.Y. Li and S.Z. Xu, 2009b. Polymorphisms of *PON1* gene digested by EcoR5 and Alu1 and its association analysis with partial traits in seven bovine breeds. J. Agric. Biotechnol., 17: 571-576.

Khersonsky, O. and D.S. Tawfik, 2005. Structure-reactivity studies of serum paraoxonase PON1 suggest that its native activity is lactonase. Biochem. (Mosc)., 44: 6371-6382.

Martin-Burriel, I., R. Osta, W. Barendse and P. Zaragoza, 1997. New polymorphism and linkage mapping of the bovine lactotransferrin gene. Mammalian Genome, 8: 704-705.

Purnell, J.Q., D.S. Weigle, P. Breen and D.E. Cummings, 2003. Ghrelin levels correlate with insulin levels, insulin resistance and high-density lipoprotein cholesterol, but not with gender, menopausal status, or cortisol levels in humans. J. Clin. Endocrinol. Metab., 88: 5747-5752.

Watzinger, N., H. Schmidt, M. Schumacher, R. Schmidt and B. Eber *et al*., 2002. Human paraoxonase 1 gene polymorphisms and the risk of coronary heart disease: A community-based study. Cardiology, 98: 116-122.

Zech, R., R.M. Severin, J.M. Chemnitius and K. Nebendahl, 1999. Paraoxonase polymorphism in rabbits. Chem. Biol. Interact., 119-120: 283-288.